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INDIAN COUNCIL OF MEDICAL RESEARCH
TECHNICAL REPORT OF THE
SCIENTIFIC ADVISORY BOARD
FOR THE YEAR 1956

Indian Council of Medical Research



TECHNICAL REPORT
OF THE
SCIENTIFIC ADVISORY BOARD
FOR THE YEAR
1956

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NOTICE

The Indian Council of Medical Research publishes annually a Technical Report of the Scientific Advisory Board. In the past detailed reports of work done by all the research schemes financed by the Council during the year were included in the Technical report. The Scientific Advisory Board, at its meeting held in December, 1956, decided that, as a measure of economy, only an abstract of the work done by the enquiries should be printed in the Technical Report. This is the first annual Technical Report in which only short abstracts of the reports of work done on the research schemes financed by the Council during 1955-56 have been printed.

C. G. PANDIT
Director

New Delhi,
July, 1957.

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- Lieut-Colonel Barkat Narain, Adviser, Community Projects Administration, New Delhi.
- Dr. P.C. Bose, Chief Engineer, Medical and Public Health Department, Government of West Bengal, Calcutta.
- Mr. O.C. Hopkins, Adviser in Public Health Engineering, Ministry of Health, New Delhi.
- Shri R.S. Mehta, Engineer and Secretary, Joint Water and Sewage Board, 7, Scindia House, New Delhi.
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- Dr. T.R. Bhaskaran, Associate Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, Calcutta. (Secretary)

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Lieut-Colonel A. N. Roy, Assistant Director of Medical Services,
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Dr. H.N. Sahai, Medical Inspector of Factories, Government of Bihar,
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Dr. B.D. Bhatia, Director, Child Guidance Clinic, College of Nursing,
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- Dr. D.W. Soman, Director, Haffkine Institute, Bombay.
- Dr. J.B. Shrivastava, Professor of Pathology and Bacteriology, Medical
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- Dr. R. Sanjiva Rao, c/o Dr. S. N. Katre, Director, Deccan College,
Poona-6.
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Dr. P. K. Sen, Professor of Surgery, Seth G. S. Medical College, Bombay.

Dr. R. P. Malhotra, Professor of Clinical Medicine, Medical College, Amritsar.

Dr. G. L. Sharma, Director of Medical and Health Services, Nagpur.

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Cholera Sub-Committee

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- Dr. M.N. Lahiri, Professor of Microbiology, All-India Institute of Hygiene & Public Health, Calcutta.
- Shri K. K. Mathen, All-India Institute of Hygiene & Public Health, Calcutta.
- Dr. E. K. Narayanan, Assistant Director, Central Research Institute, Kasauli.
- Dr. S.C. Seal, Professor of Epidemiology, All-India Institute of Hygiene, & Public Health, Calcutta.
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- Dr. N. Figueredo, Superintendent, Acworth Leprosy Home, Matunga, Bombay.
- Dr. V.R. Khanolkar, Director, Indian Cancer Research Centre, Parel, Bombay-12.
- Dr. P. L. Kapoor, Special Leprosy Officer, c/o Surgeon-General with the Government of Bombay, Bombay-1.
- Dr. N. N. Mookherji, Officer-in-Charge, Leprosy Department, School of Tropical Medicine, Calcutta.
- Dr. R. V. Wardekar, Secretary, Gandhi Memorial Leprosy Foundation, Sevagram, Wardha. (Secretary)

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- Dr. C. Chandrasekharan, Professor of Statistics, All India Institute of
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- Dr. P. Frimodt Mollar, Superintendent, Union Mission Tuberculosis
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- Dr. K.S. Sanjivi, Professor of Medicine & Physician, Madras Medical
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Dr. G.K. Karandikar, Professor of Pharmacology, Medical College, Baroda.

Dr. J.D. Pathak, Professor of Physiology, Medical College, Baroda.

(*Secretary*)

III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1956.

The researches carried out during the year under report were recommended by the Scientific Advisory Board at its meetings held in Nagpur on the 21st and 22nd December, 1955, and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 24th March, 1956. *The views expressed by the individual workers are not necessarily the views of the Council.*

CLINICAL RESEARCH

1. Clinical research unit under Dr. V.R. Khanolkar at the Tata Memorial Hospital, Bombay.

(1) *Studies in phosphatase activity.*—A study of the acid and alkaline phosphatase activity in gastric juice was undertaken year before last with a view to finding out whether the phosphatase activity has any diagnostic significance in gastric carcinoma. The study was extended to phosphatase activity in gastric mucous tissues. Results of the studies in gastric juice in the case of patients with histological evidence of gastric cancer showed that only in 4 out of the 17 samples, acid and alkaline phosphatase activity was increased above 10 K.A. Units (normal limit 0.5 K.A. Units), and that it is not a reliable index of gastric malignancy. Then, the results of work on gastric mucous tissues indicate that there is moderate increase in the acid phosphatase activity and about five-fold increase in alkaline-phosphatase activity in adenocarcinoma as compared to that in the normal gastric mucosa. It was concluded, therefore, that the changes occurring in gastric mucosa during malignant transformation are not infallibly represented by the phosphatase activity in gastric juice, probably because of its variable and complex composition.

Experimental studies on rats, however, showed that there was no significant change in phosphatase activity.

(2) Working with human smegma, which has been associated with the development of cancer of penis and of cervix uteri, three hitherto unisolated crystalline compounds, A (m.p. 80°), B (m.p. 123.5°) and C (m.p. 140.5°) have been isolated as major constituents from its petroleum-ether extract. Compound A has been shown to be an ester of β -cholestanol and compound C has been identified as β -cholestanol. The biological work so far carried out on smegma is of the exploratory nature and it indicates that the organic solvent extracts of human smegma produce organic lesions in mice.

(3) Chemical examination of chinar leaves which are used as a smouldering fuel in the Kangri pot, carried by Kashmiris under their robes for the sake of warmth during winter and suggested to caused cancer of the abdominal skin has yielded so far three waxy compounds, a sterol (probably β -sitosterol) and a β -sitosteryl glycoside.

(4) The levels of serum iron and serum iron-binding capacity in 16 control males have been studied.

(5) A comparative study of two chemical methods for the precipitation of chorionic gonadotrophic hormones is being made and a method for the estimation of the precipitated CGTH as a protein has been standardized.

2. Neuropathological unit under Dr. V.R. Khanolkar at the Tata Memorial Hospital, Bombay.

(1) In a modified approach to the experimental study of lathyrism in laboratory animals, it appears that animals that have been conditioned by the feeding of lathyrus have more damage to their nervous system than normal control animals, with the same dose of a known neurotoxic agent. These observations will have to be confirmed by further work.

(2) Observations on dietetically induced pyridoxine deficiency in monkeys are progressing and are being supplemented by similar observations on Wistar rats.

(3) Three brains from fatal cases of virus encephalitis from Vellore and five experimental animals from Delhi were examined and reported upon.

3. Hæmatological Unit under Dr. J.B. Chatterjea, at the School of Tropical Medicine, Calcutta.

I. NUTRITIONAL MACROCYTIC ANÆMIA

(A) ASSAY OF VITAMIN B_{12} .

(a) *Serum vitamin B_{12} level.*—In a series of 19 patients, the mean values of free and total vitamin B_{12} were 7.6 (range 0 to 20) and 65.6 (range 10 to 196) micromicrogram per c.c., respectively.

(b) *Urine vitamin B_{12} .*—In a series of 19 patients, the mean excretion of vitamin B_{12} was 21.2 and the range varied from 0 to 86 micromicrogram per c.c. Vitamin B_{12} was excreted in urine only in the free form.

(c) *Absorption of vitamin B_{12} with oral test dose.*—With 200 to 500 microgram, there was no detectable rise of vitamin B_{12} in serum or urine. With 1,000 to 3,000 microgram, in most instances there was a significant rise of vitamin B_{12} level both in serum and in urine.

(d) *Absorption of oral vitamin B_{12} with a combination of vitamin B_{12} plus intrinsic factor.*—Seventy-five microgram of vitamin B_{12} in combination with intrinsic factor showed a significant rise both in serum and urine.

(e) *Absorption of vitamin B₁₂ after intramuscular injection.*—Intramuscular injection of vitamin B₁₂ always resulted in high rise in serum vitamin B₁₂ level, the maximum rise being seen in 1 to 3 hours. Urinary excretion also increased very considerably.

(B) ASSAY OF FOLIC ACID.

Microbiological assay of folic acid was undertaken with a view to find out whether folic acid level in serum and in urine as measured turbidimetrically with *Streptococcus faecalis* R as test organism, could be used as indicator of folic acid deficiency. Results up-to-date indicate that serum folic acid level does not provide any dependable index in favour of the diagnosis of folic acid deficiency. Urinary excretion of folic acid following ingestion and intramuscular injection of folic acid appears to be a better indicator of folic acid deficiency.

(C) CHANGES IN SERUM IRON.

In NMA, serum iron was normal or increased. In patients showing satisfactory reticulocytic and erythrocytic response to antimegaloblastic drugs, the serum iron fell sharply within 48 hours, very often to distinctly hypoferræmic level. The drop in serum iron level usually preceded the reticulocytic response. These findings suggest that earliest indication of satisfactory hæmopoietic response could be obtained from the behaviour of serum iron level.

II. EXPERIMENTAL ANÆMIA

On milk diet, containing vitamin B₁₂ 0.05 µg., folic acid 0.8 µg. and vitamin C 1.5 mg. per litre, only one monkey out of four developed significant anæmia with fall in serum-iron level. The bone marrow showed normoblastic hyperplasia with 'giant meta-myelocytes' and 'giant stabs'. Another monkey which died on the 469th day of experiment without showing any significant anæmia showed megaloblastic bone marrow on autopsy.

III. STUDIES ON COOLEY'S ANÆMIA

Electrophoretic analysis of hæmogoblin.—Search for abnormal hæmoglobin showed high incidence of hæmoglobin E in the patients and in their family members. It appears that anæmia in these patients results from an interaction of Thalassæmia gene with that of hæmoglobin E—thalassæmia disease is clearly distinguishable from hamozygous thalassæmia only by electrophoretic analysis of hæmoglobin.

IV. STUDIES OF HÆMOSTATIC MECHANISM IN LEUKÆMIA

(a) *Chronic myeloid leukæmia.*—The significant abnormal findings were depressed plasma prothrombin activity, high serum prothrombin activity, poor prothrombin consumption, low plasma labile factor (factor V) and serum prothrombin-conversion accelerator (S.P.C.A. or factor VII).

(b) *Acute leukæmia*.—Thrombocytopenia of varying degree was a constant finding. Other abnormal findings were decreased capillary resistance, prolonged bleeding time, poor clot retraction, poor prothrombin consumption and low plasma labile factor.

V. ALKALI-RESISTANT HÆMOGLOBIN IN ANIMAL BLOOD

Alkali-resistant hæmoglobin as measured with the alkali-denaturation technique of Singer *et al.* was very high in the blood of adult animals, such as cow (mean value 78.1 per cent), goat (mean value 77.7 per cent), sheep (mean value 69.97 per cent), rabbit (mean value 69.97 per cent) and chicken (mean value 59.6 per cent). In cat and monkey the respective mean values were 6.06 and 5.7 per cent, respectively. With the same technique the mean value for adult human blood was 1.2 per cent.

4. Clinical research unit under Dr. R.N. Chaudhuri at the School of Tropical Medicine, Calcutta.

TROPICAL EOSINOPHILIA

I. AETIOLOGICAL INVESTIGATIONS.

(a) Attempts were made to reproduce the disease by the following methods, but were unsuccessful :—

- (1) Transfusion of blood from eosinophilic patients with lung symptoms to human volunteers.
- (2) Intravenous injection of blood to monkeys.
- (3) Injections of fractions of blood (plasma, red and white blood cells) to rabbits.
- (4) Tracheal instillation of sputum in monkeys from eosinophilic cases.

(b) *Field study*.—As eosinophil count above the usually accepted standard is commonly met with in this country, a survey of its incidence was made in rural areas. This was done among apparently healthy men, women and children of all ages in two districts of West Bengal. The results were as follows :—

	Group A.		Group B.
	Males	Females	Males only
Total number examined	192	138	336
Eosinophil count (per cent)			
average	15.8	15.3	12.8
Eosinophil count up to			
10 per cent	65	43	134
Eosinophil count 11 to			
19 per cent	68	65	147
Eosinophil count 20 per cent			
and above*	59	30	55

*N.B. (1) 63 per cent of group A and 90 per cent of group B belonged to the age group 15 years and below.

(2) In group A the proportion of females was about half that of males.

(c) *Familial incidence*.—Examination of blood of the family members (137) of 27 patients showed the following eosinophilia :—

	Persons
Up to 10 per cent	87
Between 10 and 20 per cent	38
Above 20 per cent	12
(one had 75 per cent eosinophilia)	

These observations are being continued.

(d) *Liver biopsy*.—This was done in ten cases of which six appeared normal histologically. The others had varying degrees of cellular infiltration of portal tracts mostly of mononuclear cells and eosinophils, the latter being also found in the sinusoids.

(e) *Sensitivity test with filarial antigen*.—Intracutaneous tests in 6 tropical eosinophilia cases using dirofilarial antigen resulted in a marked local reaction with a wheal of 20 mm. to 30 mm. diameter surrounded by erythema, appearing within 10 minutes and gradually fading away, while in the two control cases there was no reaction.

(f) *Search for spirochaetes*.—To determine the role, if any, of spirochaetes in tropical eosinophilia, the examination of mucus from the lower bowel and sputum was undertaken. Out of eighteen cases, sixteen showed spirochaetes in the bowel mucus and eight in the sputum in variable numbers. For a comparison, thirteen cases with other ailments were examined, two of them showing spirochaetes in the sputum and two in the bowel mucus.

II. CLINICAL INVESTIGATIONS.

(a) *Follow-up study*.—Eighteen cases have been followed up after arsenical therapy. At the time of discharge from the hospital the bronchial spasm had disappeared in the majority. Nine patients had no pulmonary symptoms, but simple cough persisted in an equal number except in 2 cases with unrelieved bronchial spasm, the fall of eosinophils was marked in all, irrespective of their symptoms. Radiologically, mottling or bronchial markings persisted in 5, while one case with localized crowding of shadows showed bronchiectatic changes.

After 1 to 2 years, there was all-round improvement with fourteen cases having no complaints whatsoever, and 3 only slight cough. Three patients had relapsed with cough and bronchial spasm after a period of freedom from these symptoms. There had been further fall of eosinophil count. Radiologically, broncho-vascular markings were seen in ten cases, i.e., more than on the previous occasion, but these had practically no effect on the patients except the three on relapse.

(b) *Trials with diethyl carbamazine (hetrazan) and a glucocorticoid :—*

(i) Hetrazan was tried in 28 patients with an average eosinophil count of 16,634 per c.mm. Hookworm was present in 3 patients, *Giardia* cysts in 5, and trichuris in 1, but none had microfilaria in the blood or ascaris in the stools. The eosinophil count came down below 2,000 per c.mm. with relief of lung symptoms and radiological improvement in twenty cases. The results were indeterminate in five cases and 'failures' in three. The response of tropical eosinophilia to hetrazan and arsenic suggests some underlying infection or infestation.

(ii) Deltacortril brand of Prednisolone was tried in five cases. There was good response clinically and hæmatologically, and also radiologically in some. The eosinophils were reduced to normal limits within a week in most of them, but soon started rising with recurrence of symptoms and re-appearance of radiological mottling. The response to deltacortril is suggestive of an allergic process in the ætiology.

CHRONIC SPLENOMEGALY

From a study of 161 apparently idiopathic cases admitted to the hospital during the last 3 years, it appears that they are of varied ætiology and can be divided into the following groups :—

Classification :

(1) Tropical splenomegaly (with history of recurrent malaria in the past)		134
		(All but 3 cases belonged to rural areas)
(a) Cases responding to suppressive antimalarial therapy	37	
(b) Cases usually with little or no response to suppressive anti-malarial therapy.	86	
(c) Cases with terminal portal cirrhosis.	11	
(2) Portal obstruction with early gastro-esophageal varix.		16
(a) Intra-hepatic (primary cirrhosis)	10	
(b) Extra-hepatic	6	
(3) Other cases		11
(a) Cooley's anæmia	5	
(b) Sequel to infective hepatitis	6	

As shown above, by far, the vast majority of the cases were of malarial origin, and this report is chiefly concerned with them as tropical splenomegaly. The hypothesis of malaria as the initial cause is supported by many facts. These patients came from malarious places, had suffered

from malaria for long period in the past, and many had irregular fever off and on after admission to hospital. If not advanced they responded well to suppressive anti-malarial therapy for several months with reduction of spleen and liver, but with the development of 'hypersplenism' or over-active spleen leading to pancytopenia the response was poor except in some cases and that too after very prolonged therapy (2 to 3 years). This is also well illustrated by our experience in rural areas, where as a result of antimalarial prophylaxis carried out for 6 successive years, the spleen rate and size became remarkably reduced in many cases. It is true that malarial parasites were not commonly found in the hospital patients, but this is not unusual in chronic cases, and it is noteworthy that *vivax* parasites appeared in the blood of nearly 40 per cent of the patients who were subjected to splenectomy. In this connection mention may be made of our experiments on rats, which tended to show that latent infection with *P. berghi* can cause marked enlargement of the spleen.

There is already enough evidence to show that malaria may cause degenerative changes in the liver, and that such changes are specially predisposed when there is accompanying malnutrition. In the experiments just referred to, it was observed that the latent malarial infection could also cause hepatic damage and that this damage became severe when the diet was protein deficient. Progressive disappearance of malarial pigment was also noticed. These conditions and changes resemble, to some extent, the findings observed in many of our patients.

The chief characteristics of this group of tropical splenomegaly were massive enlargement of the spleen, and to a less extent of the liver with varying degrees of anæmia in the majority of cases. The spleens removed after operation showed marked proliferation of reticulo-endothelial cells, congestion of sinuses and fibrotic changes. Liver biopsies in 65 patients revealed all grades from 'no significant pathological change' to 'marked cirrhosis'.

The classification of these cases into 3 sub-groups was made according to residence, history, laboratory investigations, liver changes, and effects of prolonged suppressive anti-malarial therapy in the out-patients clinic and in the field. Briefly the chief characteristics were :—

1 (a) *Chronic splenomegaly responding to antimalarials*— 37 cases. These were uncomplicated cases of chronic malaria with no obvious liver changes, or at most round cell infiltration of the portal tracts. Suppressive anti-malarial therapy once a week or even a fortnight for 5 or 6 months was sufficient to cure them.

1 (b) *Chronic splenomegaly with 'hypersplenism'*— 86 cases. It seems that patients with chronic and latent malaria superimposed on malnutrition state of health may arrive at a stage when hypersplenism develops, in which anæmia becomes a marked feature, as it did in many cases of this group. Liver biopsy in 40 cases showed areas of focal necrosis and scattered fibrosis, fatty changes in some cases, round cell infiltration of portal tracts, and proliferation of Kupffer cells. Deposits of iron pigment were not infrequently encountered. There is little or no response to anti-malarials, but prolonged observation in villages has

shown that suppressive therapy may be useful in some cases if continued especially during the transmission period for 2 to 3 years. Anti-anæmia treatment is very useful in this sub-group, though it has often to be repeated.

1 (c) *Chronic splenomegaly with terminal portal cirrhosis*—11 cases. This is a late stage of the previous sub-group, of which the picture is that of portal cirrhosis with more or less marked portal hypertension. Treatment is only symptomatic.

It is possible that splenomegaly like the cases under 1 (b) and (c) might be a sequel to infection other than malaria or due to as yet undetermined causes.

Treatment.—Antimalarials, hæmatinics and splenectomy in some resistant cases were the main lines of treatment. Follow-up of a limited number of patients seemed to show that it was the condition of the liver which determined the response to treatment. Liver biopsies of some cases in 1 (b) repeated serially for two years revealed aggravation of the original pathological lesions—marked in 2 cases. Three patients who responded well to treatment with insignificant change in initial biopsy specimen showed no changes, however, after 1 to 2 years. Three cases in 1 (c) died of hepatic failure after hæmatemesis, one of them showing post mortem a cirrhotic liver. The immediate effects of splenectomy in 14 cases of 1 (b) were good, the patients gaining weight with return of the normal blood picture except leucocytosis (often with eosinophilia), persisting for a fairly long time, but this was not maintained usually for more than a year. Eight of these cases were followed up for 2 to 2½ years; one died of cirrhosis with ascites, another had melæna, two had pain in the liver and jaundice, while three had repeated attacks of *vivax* malaria despite specific treatment. Only one is doing well. Liver biopsies showed changes in most of them. Experience shows that splenectomy is indicated only in cases of massive splenomegaly with severe pancytopenia not showing sustained improvement with antimalarials and hæmatinics, provided the liver biopsy does not show significant pathological changes. Gross change of hepatic parenchyma is a definite contra-indication to splenectomy.

SPLEEN EXTRACTS

Two extracts, alcoholic and acetone, were prepared and are being tried in persistent urticaria and some other conditions.

5. Liver Diseases Research Unit under Dr. P.N. Wahi at the S.N. Medical College, Agra.

I. *Adrenal cortical function in human liver disease.*—In view of the experimental evidence obtained in our earlier study that in carbon tetrachloride-induced cirrhosis in rats, the irreversible stage of the cirrhosis is characterized by stunted adrenal function, while in the reversible stage the adrenal function is normal (*Annual Report*, 1955;

Wahi *et al.*, *Arch. Path.*, **62**, p. 215, 1956), it was thought of interest to study the adrenal cortical function in clinical cases of portal cirrhosis and infectious hepatitis. Eisenberg *et al.* (*Gastroenterology*, **18**, p. 36, 1951) and Peterson *et al.* (*Jour. Lab. Clin. Med.*, **43**, p. 58, 1954) have reported decreased urinary 17-ketosteroid excretion in clinical cases of liver disease and have taken this to indicate impaired adrenal function in these cases.

In the present study, adrenal-cortical function was assessed by a battery of adrenal-function tests as follows :—

(1) Blood eosinophil count before and after intramuscular ACTH administration. (2) Uric acid and creatinine estimation in urine before and following ACTH administration. (3) Hyaluronidase-fluoresce in skin-wheal test. (4) Urinary 17-ketosteroid excretion. Simultaneously, the liver function tests were also performed and a detailed clinical history was taken in each case. In this way, 9 cases of portal cirrhosis and 12 cases of infectious hepatitis were studied. It was found that on the basis of the response to these tests there was impaired adrenal function in 7 of the cases of portal cirrhosis and 8 of the cases of infectious hepatitis.

II. *Study of the pattern of amino acid excretion in liver disease.*—There is considerable evidence in the literature that nitrogen metabolism is disturbed in liver disease (Eckhardt *et al.*, *Trans. N.Y. Acad. Sc.*, **10**, 284, 1948; Dunn *et al.*, *Jour. Clin. Invest.*, **29**, p. 302, 1950; Dent and Walshe, Ciba foundation Symposium on Liver Disease, 1951). Having evolved a standard technique of paper-partition chromatographic analysis under our climatic conditions and prepared a comprehensive standard chart of the R_f values of amino acids, a detailed study of urinary amino acid excretion patterns in portal cirrhosis and infectious hepatitis was carried out. At first, the normal pattern for individuals in this part of the country was established. The normal pattern was found to consist of four amino acids, i.e., glycine, serine, alanine and glutamine. Following the same technique, 9 cases of portal cirrhosis and 12 cases of infectious hepatitis were studied. It was observed that 8 of the cases of portal cirrhosis and 9 of the cases of infectious hepatitis showed an abnormal pattern, involving an increased excretion of one or more of the following amino acids : Cystine, methionine, glycine, serine, alanine and glutamine. In a few cases the quantitative estimation of the amino acids was also carried out using the technique previously described (Wahi *et al.*, *Ind. Jour. Med. Sc.*, **8**, p. 427, 1954).

III. *Study of the role of the adrenal cortex in the histogenesis of dietary cirrhosis in rats.* As a corollary to our earlier study on the role of the adrenal cortex in carbon tetrachloride-induced cirrhosis in rats, a total of 130 rats was put on a high-fat low-protein diet for a number of months and the adrenal cortical function studied by the ascorbic acid depletion method at the different stages of the evolution of the cirrhosis. Fatty infiltration of the liver was observed at end of 9 weeks and the earliest signs of fibrosis after 11 weeks on the diet. None of the rats, however, survived till the onset of full-fledged cirrhosis. Preliminary

data indicate that onset of the fibrosis causes progressively lesser depletion of the adrenal ascorbic acid indicating there is damage to the adrenal. The work is being continued.

IV. *Liver damage in congestive heart failure.*—A comprehensive study of clinical, biochemical and histological aspects of changes in liver in different etiological types of congestive heart failure was done in 61 cases. The liver damage was assessed by a battery of liver function tests. Liver biopsy material was studied in 29 cases, including 4 post-mortem cases. Histological changes varied from earliest centrilobular necrosis and congestion to well-advanced cardiac fibrosis. Paper partition chromatographic analysis of urinary amino acids indicated a marked amino aciduria in a number of cases.

V. *Liver function studies in leprosy.*—A total of 52 cases was investigated. Of these 13 were of the lepromatous type and the rest of neuromacular and tuberculoid types. Of the 13 of the first type, flocculation tests were abnormal in six but there was no correlation with age and sex of the patients, duration of the symptoms and severity and distribution of the lepromatous nodules. None of the other liver function tests were abnormal. In the neuromacular and tuberculoid group, only four showed evidence of deranged liver function; but here again there was no obvious correlation between clinical picture, duration of disease and functional damage.

VI. *Histopathological changes in liver in chronic amœbiasis.*—Twenty-nine liver biopsies were studied for histological changes in liver in chronic amœbiasis. Most of the sections showed a diffuse parenchymal damage seen as obscured cellular outlines, rarefaction and fragmentation of cytoplasm and eosinophilic smudging. In 21 sections there was evidence of focal necrosis and in 2 cases mild degree of fatty infiltration was present. The most conspicuous change was the reticulo-endotheliosis.

VII. *Registry of hepatic pathology.*—A total of 452 cases has been registered in the hepatic registry till date. The important contributions during the year were from Dr. P. Malkani and Dr. H.F. Smetana on infectious hepatitis, Dr. J. Gillman on nutritional dystrophy and Dr. M. Paic (Rio de Janeiro) on yellow fever and infectious hepatitis. The registry was visited during the year among others by Dr. Hans Smetana (Washington—Delhi), Dr. Edith Potter (Chicago) and Dr. Edmonds (Fuji Islands).

The bibliographic index of articles on liver diseases in different journals available in India is being maintained and cyclostyled copies of the index up to 1955 have been circulated.

6. Inquiry on the endemic focus of schistosomiasis in India at the Grant Medical College, Bombay.

Dr. N. Ansari, Head of the Endemo-epidemic Diseases Section of the W.H.O., Geneva, visited the inquiry during November-December,

1955. He went through the work as carried out in this Laboratory. He was taken to Poona and then to the village Gimvi—the endemic focus of schistosomiasis in India.

Antigen preparation.—The emission experiments on the naturally infected *Indo-planorbis exustus* were carried out to isolate those snails emitting only brevifurcate furco-cercariæ. Such snails were dissected and the livers of those which were heavily infected (numbering 30) with brevifurcate furco-cercariæ were taken out, dried under negative pressure, powdered, sterilized and sealed in an ampule. Also, 30 livers from non-infected snails were powdered and sealed in another ampule similarly to serve as control.

Infection of snails.—Over a thousand *Ferrissia tenuis* were infected with Gimvi strain. Mass infection was given to about 1,000 snails (*F. tenuis*) in an aquarium. Batches of 50 snails, i.e. *Indo-planorbis*, *Limnæa* and *Planorbis*, were infected at three different times. A mass infection was also given to *Indo-planorbis*, *Limnæa* and *Planorbis* in the aquaria which they contained. Recently, mass infection was given to about 60 young *Limnæa* and 30 young *Vivipara*.

The number of ova in the urine of the patient available in Bombay, was considerably fluctuating. The viability of ova was affected sometimes. At another time the miracidia that hatched out were not so active as required for infection of snails. No frank hæmaturia case was available either at Bombay or at Gimvi, as required for infection experiments. At Gimvi, all the patients were recently treated with Fantorin by the Public Health Department, Poona. During the current year, the snail population at Gimvi was also reduced, though not eradicated by the treatment of the stream with copper sulphate by the Public Health Department, Poona.

The infected *Ferrissia*, that survived a period of five weeks after the infection, emitted few cercariæ. A yield of 50 cercariæ was available time and again. The number of cercariæ was too less for the animal infection. As a result of that the infected animals were observed to be negative after a period of three months. It may be noted that nearly a minimum of 500 cercariæ are needed to infect a laboratory animal. Few infected *Indo-planorbis* and *Limnæa* that survived a period of infection were observed to be negative. *Planorbis* were also negative.

One of the reasons why schistosome infections are not common in India is that *Ferrissia* is not a good host capable of prolific liberation of cercariæ. This infection will, therefore, only be confined to pools loaded with *Ferrissia* and getting chance of being infected. This will usually lead to a sporadic infection except in a locality, such as Gimvi, where conditions for continuation of cycle are ideally established in course of several years past. Even in Gimvi, the treatment of the infected, treatment of water and improved water supply will very likely lead to wiping out of the endemicity. However, so long as the snails are not wiped out completely and especially if they are not vigilantly got rid off or kept in check, the infection may continue as a sporadic infection in the village. Such sporadic cases have come to our light from Malsana

in Nasik district, Mudh-island in Bombay, village Vasarana in Dangs district and Gogi in Gulberga. Dr. Ansari also quotes cases from Delhi and Punjab as mentioned by Dr. Dhanda. Schistosomiasis is thus a widespread Indian sporadic infection and both faecal and urinary infections occur. The question of its having adapted to other strains of snails besides *Ferrissia* as in Gimvi has to be considered. Gimvi has offered an ideal spot for study, while in other localities, due to singularity of a case, such investigations have borne no fruit. We have carried out surveys in Malsana, Mudh and Vasarana but the results are not such as to give any clue for the above reason. It is proposed to survey Gogi in the near future.

Emission experiments on the snails, collected from the suburbs, were carried out to get different types of bifurcate *carcaria* (animal schistosome) for the infection of the laboratory animals.

Animal infection.—A number of animals, mice, guinea-pigs, hamsters, rabbit and pigeons, were infected with Gimvi strain, Ahwa (Dangs) and animal schistosome strain.

Adult worms.—About 100 adult worms, *Schistosoma spindalis*, were recovered from laboratory-infected animals. Experiments on fixation and mounting of adult worms with lacto-phenol were carried out with satisfactory results.

An epizootic in the laboratory carried away a number of animals including those infected with Gimvi strain as well as Dangs strain.

Animal faeces.—Animal faeces samples collected from the suburbs and the laboratory were examined for ova with negative results.

Study of ova.—The observations on the measurements of ova (*S. hæmatobium*) were confirmed and tabulated.

Gimvi survey.—The survey of snail population throughout the infected stream invading the village Gimvi was done. It is observed that the population of *Ferrissia tenuis* as well as that of *Paludomus obesa* was considerably affected in this season, since the treatment of the infected stream by the Public Health Department, Poona. They must pursue these efforts every year after October and up to March and also train up their personnel in recognizing these snails on dried, decaying mango-leaf as it is of negligible size which could be easily overlooked by an untrained eye. The eradication of this snail is not an easy problem. Reduction of snail population and incidence of snail infection in the locality below a critical level is perhaps easy. The public health vigilance should continue at least for 5 years and every case recorded during this period in the locality must be dealt with vigorously.

The survey of the percentage of (*F. tenuis*) infection was also done. The percentage of infected snails, in general, varied between 0.7 per cent and 2.0 per cent in the stream, as a whole, and at one exceptional spot the percentage of infected snails went up as high as 15 per cent.

The urines of the suspected patients (school children) were examined. But no case with frank hæmaturia was found, as the patients were recently treated by the Public Health Department, Poona. A few patients showed few ova but these ova were coated with a calcareous coat and were not viable. Thus, we were unable to get a patient with frank hæmaturia for our laboratory experiments.

Dangs survey.— A case of *Schistosoma hæmatobium* was reported in 1948-50 from Ahwa (Dangs). Hence, a general survey of that area was carried out in order to trace out that case and also to study the snail fauna and the type of infection.

The following snails were collected from the survey of five rivulets : (i) *Vivipera bengalsnsis*, (ii) *Indo-planorbis exhustus*, (iii) *Limnaea luteola*, (iv) *Planorbis torquis*, (v) *Melenoides species 1*, (vi) *Melenoides species 2*, and (vii) *Paludomus obesa*. *Ferrissia tenuis* was not detected in this locality. This finding is similar to the one obtained in Malsana and leads us to suspect that snails other than *Ferrissia* may have been adapted as intermediate hosts in some localities. We have persistently failed, however, in infecting other snails except *Ferrissia*. The efforts to adapt the infection in other snails are being continued.

From the case papers from the Central Hospital, Ahwa, the patient's address was traced and the urine examinations of the inhabitants of that village (Vasarana) carried out. They were all negative. The reported patient died in 1950. Urine examination of some suspected cases was also done in the Central Hospital, Ahwa, but with negative results.

Human Schistosomiasis in India. Parts I, II, III and IV have already appeared in the *Indian Journal of Medical Research*.

7. Inquiry on the study of Indian poisonous snakes under Dr. P.J. Deoras at the Haffkine Institute, Bombay.

During the current year, attention was devoted to collecting food for rearing young one of snakes. It was noticed that the tadpoles of four distinct species were available in different months. These being *Rana tigrina*, *Rana limnocharis*, *Rana rufescens* and *Rana brevicep*. Thus, young frogs were available for young snakes up to October. Venom output studies have shown that the cobra snakes have been giving more venom in the farm constantly for the last three years but the output of venom in Russell viper and krait snakes were varying. The mortality of snakes in the farm was less than those in the room. These are being analysed statistically. The morphological studies of *Echis carinatus* have been completed. These studies for the four snakes have shown that sexual colour variations occur during summer month in Russell vipers. Similarly, the spots on this snake available in the North are different from those in South India. They differ in size, ferocity and even in venom output though designated as one species. *Echis* collections have shown a few specimen without the routine spot marks. In cobra the head mark

varies from mere two parallel bars to a complete U shape. In krait the young ones are pinkish with the anterior bars as mere white spots and could often be mistaken for *Lycodon* sp. In the internal anatomy, respiratory and urinogenital system show very marked differences. The right lung is largely enlarged in all these snakes, while the left is absent in Russell's viper, nearly absent in echis and tiny rudiments in krait and cobra. The male reproductive system in Russell viper has a U shaped bifurcated penis on either side studded with soft suckers and spines. In echis there is slight bifurcation but in krait and cobra there is no bifurcation.

Conclusion.—These morphological figures are being drawn and when completed they would throw some light on the systematics of these snakes and make available the knowledge of the internal structure which was little known so far.

8. Enquiry into the changes—Clinical and Pathological—that are present months or years after apparent recovery from 'Kwashiorkor' or 'Nutritional dystrophy' under Dr. S.T. Achar at the Government General Hospital and the Madras Medical College, Madras.

27 children who had Kwashiorkor some years previously and who were examined in previous years from time to time were re-examined this year. Only one showed pronounced hepatomegaly with definite and marked increase connective tissue proliferation as compared to previous years. There have been 2 new cases of pronounced hepatic fibrosis of the type nutritional, without previous history of Kwashiorkor similar to the ones encountered and reported in previous reports. The possible role of dietetic status on the sequelae of infective hepatitis in children is deduced from a study of a series of 170 post-infective hepatitis syndrome cases with regard to their dietetic history, clinical condition and liver histology.

9. Inquiry into the use of artificial hypothermia (hibernation) in open intra-cardiac surgery under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

Fifty experiments have been carried out since 1st October 1955. This work has been divided into three main heads :—

1. *Studies in extreme hypothermia.*—To carry out open 'dry' cardiomyotomies on completely non-pulsatile, 'stand still' heart with a view to exclude cerebral anoxia, ventricular fibrillation, coronary flow, and air embolism. A preliminary group of 18 experiments to study the behaviour of the heart to low temperatures ranging from 15°C to 9°C. were conducted.

2. A further group of 17 experiments was conducted in order to study the behaviour of the isolated heart lung with intact coronary and pulmonary circulation with a view to complete homologous heart lung transfer from one animal to another at a later date.

3. A group of 7 controlled experiments conducted to study the methods of prevention and treatment of ventricular fibrillation occurring during low temperature cardiomyotomies.

Eight experiments have failed due to various technical errors.

EXTREME HYPOTHERMIA

Open cardiac surgery under hypothermia is limited primarily by the short period for which heart can be excluded from the circulation without causing permanent damage to the brain. Also, the cold heart is very susceptible to ventricular fibrillation during the operative procedures. Further, it is virtually impossible to completely remove the trapped air from the cardiac chambers during open cardiomyotomies in a beating heart by filling them with fluid. When a profound degree of deep hypothermia is introduced to produce arrest of the motions of the heart and lungs greater technical accuracy is attainable. With these objects a group of preliminary 18 controlled experiments have been carried out in the present series.

Experimental procedure.—Adult mongrel dogs were anaesthetized with intravenous pentothal (30 mg./kg. body-weight) and endotracheal tube was inserted and were hyperventilated (48 times per minute). Electrocardiographic control readings were taken and animals were immersed into ice-cooled water after recording the original temperature with a rectal electric thermo-couple. The rectal temperature and e.c.g. records were maintained every five minutes. Tables I and II show the behaviour of the 18 animals in detail. When the desired lower temperatures were obtained the cooled water was siphoned off and replaced by tap-water. Gradually, the temperature of bath was raised to 45° C. to re-warm the animals to their original respective temperatures.

It was found that out of 18 dogs, 7 went into complete cardiac arrest (Nos. 284, 288, 292, 294, 296, 298, 302). Four dogs (Nos. 284, 288, 294 and 298) did not record heart beats on re-warming. Rest of all the animals have revived and these include three dogs (No. 292, 296 and 302) who have gone into complete cardiac arrest.

ISOLATED HEART-LUNG PREPARATION

The problem of transferring the whole heart with lungs from one animal to another presents many problems of great complexity of which only one is the cessation of circulation that must follow during transfer in the organism. Our researches with hypothermia seemed to provide an answer to this difficulty as in the state of hibernation the brain can withstand stoppage of circulation for much longer period.

METHODS AND MATERIAL

The first stage consisted of removing an intact heart-lung preparation whose circulation and economy were interdependent and were together self-supporting as it were. Adult mongrel dogs were used throughout. Anaesthesia was induced with pentothal and animals were hyperventilated and temperature lowered from normal 36°C — 37°C to 27°C . A sternum cutting transverse incision opened the chest through 5th interspace. The internal mammary arteries were ligated and cut. A rib spreader is introduced. Superior vena cava is freed, pericardium is opened and aorta is freed from anterior pad of fat which hides most of aorta. The inferior vena cava is dissected. The cardiac lobes of lungs are freed from diaphragm and plane of separation extended to behind the heart. The heart is rotated forward, clamping the bronchial arteries. The azygos vein is also ligated and divided. Now the separation is begun. The vena cava are ligated and divided, a clamp is put across the terminal part of ascending aorta, 1 c.c. of 1.2 per cent KCl is injected in left ventricle for perfusion through coronaries and aorta is divided. It is possible to dissect in facial plane and detach entire specimen after dividing the nerves and trachea. Another intra-tracheal tube is passed and heart-lung put on towel and direct e.c.g. readings are taken.

Studies on the isolated heart-lung preparation.—It was found from our previous 10 experiments as (reported in previous report) that ventilation of the isolated heart-lung preparation remained more than adequate as the specimen remained pink including the heart and coronary arteries. In the previous experiments, the period of survival varied from 9 minutes to 35 minutes and period of effective beats continued for average of 10 minutes. It was noted that electrocardiograms taken by direct cardiac leads showed some injury pattern in almost all cases. Since inversion of the T wave is a usual accompaniment of deep hypothermia, the T wave changes could not be regarded as significant; but in most specimens the T wave which had become upright after injection of potassium in the left ventricle prior to removal of the specimen became gradually flattened and ultimately inverted as the beats in the isolated specimen became weaker and heart grew more flabby.

In this series of 17 experiments as seen from Table III the period of survival varied from 10 minutes to 210 minutes (dog No. 14) and period of effective beats varied from 5 minutes to 110 minutes. Hence, both the total period of survival and effective beats have increased considerably for the safe transfer of isolated heart lung preparation. The electro-cardiographic changes resembled our previous preliminary experience. The effect of various drugs, such as calcium gluconate, adrenaline (1:20,000), 1.2 per cent potassium chloride, prostigmine (1:4,000) and digoxine has also resembled our previous experience.

CONTROLLED EXPERIMENTS

As reported previously, it was found that ventricular fibrillation accounted for about 2/3rds of the total mortalities. After perfecting a

standard method of production of ventricular fibrillation the present series of experiments were conducted to determine the effect of various drugs in prevention of artificially created ventricular fibrillation under artificial hibernation.

The method used is as follows : The temperature of adult mongrel dogs was lowered to 27°C . by our standard method of induction of hypothermia as reported in previous experiments. A 5th intercostal left thoracotomy was performed after taking e.c.g. readings constantly. The venæ cavæ and aorta were clamped and drug to be tested was injected into left ventricle for perfusion through coronaries and standard duration of 4 minutes allowed in all cases for isolation of heart and induction of anoxia and at the end of which fibrillation was created by injection of 3 c.c. of 1:20,000 adrenaline into right ventricle. The continuous e.c.g. records are taken to study behaviour of heart during controlled experiments.

Table IV shows that in 6 experiments 2 c.c. 1.2 per cent KCl was injected in left ventricle and fibrillation developed in 3 which all could be revived back to normal sinus rhythm after massage, while in one experiment when prostigmine (1:4000) 2 c.c. was injected ventricular fibrillation did not produce. Our previous experiments were classed under 10 groups :—

1. Normal ventilation with air.
2. Normal ventilation with pure oxygen.
3. Hyperventilation with air (48 times a minute).
4. Hyperventilation with intravenous KCl.
5. Hyperventilation without surgery and without iv. fluids.
6. Normal ventilation with intravenous KCl.
7. Hyperventilation with glucose and KCl intravenous.
8. Hyperventilation with only glucose.
9. Hyperventilation with intracardiac KCl of various percentages in left ventricle after clamping aorta (2 c.c.).
10. Hyperventilation with intracardiac prostigmine 1:4,000 (2 c.c.).

It is obvious from the present experiments that potassium (1.2 per cent isotonic) and intracardiac prostigmine (1:4000) perfusions of coronaries for four minutes before cardiac irritation helps by preventing the onset of ventricular fibrillation.

TABLE I

Dog number	Lowest temperature, °C.	Lowest heart rate per minute
283	15	6
284	9	Cardiac arrest
287	13.5	10
288	15	Cardiac arrest
291	15	10
292	13	Cardiac arrest
293	12	7
294	12	Cardiac arrest
295	12	10
296	12	Cardiac arrest
297	9	5
298	10	Cardiac arrest
299	12.5	4
300	12	4
301	12	4
302	13	Cardiac arrest
304	12	6
305	11	7

TABLE II

Dog number	DURATION OF COOLING IN		Duration below 10 beats/minute	Survival
	Hours	Minutes		
283	2	5	25 minutes	Yes
284	1	55	Cardiac arrest	No
287	2	15	15 minutes	Yes
288	1	55	Cardiac arrest	No
291	1	40	15 minutes	Yes
292	1	25	Cardiac arrest	Yes
293	2	50	1 hour	Yes
294	2	55	Cardiac arrest	No
295	2	15	1 hour	Yes
296	2	5	Cardiac arrest	Yes
297	2	30	105 minutes	Yes
298	3	10	Cardiac arrest	No
299	2	30	90 minutes	Yes
300	2	30	100 minutes	Yes
301	2	40	120 minutes	Yes
302	2	30	Cardiac arrest	Yes
304	2	15	45 minutes	Yes
305	1	45	20 minutes	Yes

TABLE III

Behaviour of isolated heart-lung

Serial number	Survival, minutes	Rhythmic beats, minutes	Effective beats, minutes
1	45	20	5
2	25	25	5
3	45	35	15
4	45	10	10
5	50	45	45
6	180	110	110
7	20	18	18
8	10	10	10
9	57	23	23
10	50	35	35
11	35	35	30
12	35	35	35
13	70	60	38
14	210	95	95
15	110	60	50
16	50	45	45
17	85	80	80

TABLE IV
Controlled group of experiments

Dog number	Type of variation	V.F. developed	RESUSCITATORY MEASURES ADOPTED				Reversion to normal rhythm.	Remarks
			Massage	Electric defibrillation	Intracardiac KCl 1.2 per cent	Intracardiac prostigmine 1 : 4,000		
275	1.2 per cent KCl in L.V. 2 c.c.	Yes	Yes	...	...	Yes	Yes	...
276	1:4,000 prostigmine in L.V. 2 c.c.	...	...	...	...	...	...	Prevention of V.F. only low voltage in e.c.g. Irregular rhythm.
277	1.2 per cent KCl in L.V. 2 c.c.	...	...	...	...	...	...	...
278	1.2 per cent KCl in L.V. 2 c.c.	Yes	Yes	...	...	...	Yes	...
280	1.2 per cent KCl in L.V. 2 c.c.	...	...	...	...	...	...	Prevention of V.F.
281	1.2 per cent KCl in L.V. 2 c.c.	...	...	...	...	...	...	Prevention of V.F.
282	1.2 per cent KCl in L.V. 2 c.c.	Yes	Yes	...	...	...	Yes	...

V.F.—Ventricular fibrillation.

L.V.—Left Ventricle.

10. Effect of metaphyseal stimulation of longitudinal bone growth under Dr. B. Mukopadhyaya at the P.W. Medical College, Patna.

The experiment carried during the years 1954 and 1955 indicated that placement of autogenous cortical bone graft in the region of the metaphysis of young goats produced a temporary acceleration of growth in the operated limb. This acceleration was short-lived. The increase in growth, obtained as a result of the acceleration, was not maintained so that ultimately by the time the growth in the limb ceased to occur, the two limbs once again became equal in length. It was thought that the temporary nature of this growth acceleration might be the result of the insertion of autogenous graft taken from young growing animal whose grafts were fragile and were easily absorbed. In order to make the process of stimulation more sustained, hard cortical adult bone grafts were inserted in the region of the metaphysis in the present series of experiments. The work that has progressed so far does not permit any definite or even tentative conclusion to be drawn as the grafts that were inserted in the first series of animals operated upon has not yet been fully absorbed.

11. Studies on antifatty liver fractions of the pancreas and their scope in the prevention and treatment of fatty livers under Dr. J.C. Sachdev at the M.G.M. Medical College, Indore.

It has been demonstrated previously that intraperitoneal injections of ethionine caused a definite increase of liver lipids in albino rats, while antifatty liver factor of the pancreas and lipocaic when administered simultaneously with ethionine failed to prevent fatty infiltration. Methionine, however, on the other hand, could prevent this accumulation under similar conditions.

Preventive action of antifatty liver fraction and lipocaic on fatty livers induced by intragastric administration of ethionine and casein in pancreatic duct ligated rats was further studied in 25 normal female fasted albino rats weighing between 100 g. and 200 g. It was observed that ethionine fatty liver could be induced by intragastric feeding of ethionine in pancreatic duct ligated rats and that antifatty liver factor and trypsin can prevent such fatty livers in these animals. Lipocaic was not effective even by this route in these animals. From these results it could be concluded that antifatty liver factor and trypsin owe their action probably to their ability to liberate methionine from the ingested food and that lipocaic does not possess this action.

In order to elucidate that the preventive action of antifatty liver fraction is not dependant upon these fractions reaching the target organ in higher concentrations by this route, further studies were undertaken, where the preventive action of the antifatty liver fractions, and lipocaic were studied on fatty livers induced by intraportal administration of ethionine in these animals. For this work 25 female albino rats with an average weight of 140 g. were divided into 5 groups as in the previous study. Ethionine dissolved in warm saline was administered

in 100 mg. doses intraportally. Supplements of methionine, lipocaic and antifatty liver fractions were administered in 50 mg. doses along with ethionine.

Animals receiving ethionine alone, by this route showed an increase of total liver lipids. An average value of 10.8 per cent was obtained in these animals. No increase in liver lipids was observed in animals receiving saline only and those receiving a supplement of methionine. Animals in groups which received supplements of lipocaic and antifatty liver factor showed a definite increase of total liver lipids, average value being 12.38 and 12.16 per cent, respectively.

12. Inquiry on the development of ascites and its relation with portal vein pressure and blood flow in hepatic artery under Dr. R.M. Mehrotra at the K.G. Medical College, Lucknow.

The role of portal hypertension in the pathogenesis of ascites which develops in carbon tetrachloride cirrhosis of albino rats was studied during the period of this report. Cirrhosis was produced in 86 healthy adult albino rats by repeated biweekly intramuscular injections of carbon tetrachloride in doses of 0.1 c.c. per 100g. of body-weight extending over a period of 29 weeks. The animals were sacrificed at varying intervals and the incidence of ascites recorded. At the time of killing the animals, pressures in the portal vein were taken by a specially devised null-point water manometer. Histological examination of liver was also carried out.

All the animals given CCl_4 developed cirrhosis which was graded into three groups depending upon the degree of gross and microscopic changes in the liver.

The results showed that ascites was commonly observed in animals with grade III cirrhosis and that none of the animals with early cirrhosis developed ascites. The portal vein pressures were raised in the majority of the animals. The increase in the portal vein pressure was not found to bear any relation to the amount of ascitic fluid. There were also instances where the portal vein pressures markedly raised, the degree of fibrosis well advanced yet ascites was absent. It was concluded on the basis of the results of this investigation that increase in the portal vein pressure is not the sole cause for the development of ascites.

13. Studies on heart in anæmia under Dr. R.P. Malhotra at the Medical College, Amritsar.

The purpose of the present study was to investigate the cardiovascular system of patients with reversible anæmia of different types and grades by comparing observations made before and after treatment, and to assess the significance of abnormal features. A total number of 50 cases had to be done. The work was started on 28th July, 1955. In all 48 cases were taken up till 30th September, 1956. Three patients are still in the hospital under treatment and 42 cases have been discharged

so far with the advice to continue with the treatment and report for follow-up. Nine patients were females and thirty-three were males. The various investigations, such as complete hæmogram, urine, and stools examination, gastric analysis, blood proteins, electro-cardiographic studies, radiological studies, venous pressure, circulation time, etc., were carried out in each patient and repeated at suitable intervals. Forty patients suffered from microcytic anæmia, 2 from macrocytic anæmia, 20 patients from ankylostomiasis, 1 from chronic malaria, 6 from internal piles, 5 from internal piles and ankylostomiasis, 3 from post partum hæmorrhage, 1 from repeated pregnancies, 1 was refractory, and 5 from chronic diarrhoea (it is proposed to drop out 5 cases of diarrhoea from this analysis and to complete the investigations of 50 cases without any associated nutritional deficiency). In 24 patients, the hæmoglobin was below 5 grammes, in 10 patients it was between 5 and 7 grammes, in 8 cases it was between 7 and 9 grammes. Fifteen patients had no free acidity, 27 had normal acidity in the gastric juice. These were all cases of reversible anæmias. They were treated with ferrous sulphate by mouth I/V iron (Uniferon) by intravenous injections and blood transfusions. Venous pressure, x-ray heart size, e.c.g. were repeated after every blood transfusion. Hæmoglobin, and red blood cells were repeated after every week. Hæmograms were repeated after every fortnight. All these patients were discharged when their hæmoglobin was above ten grammes and then they were asked to come after every month for follow-up. These observations were repeated at the time of follow-up during which period all the patients received specific anti-anæmic therapy.

CONCLUSION

The work is still continuing, but the results so far obtained are as follows :—

Venous pressure.—In the present series, a fall in venous pressure occurred with treatment in all cases except a few and was most striking in patients with a very much raised venous pressure at the beginning.

Circulation time.—This showed variable figures, and relevant analysis has still to be done.

X-ray heart size.—The heart size was enlarged at the time of admission but became normal with the subsequent treatment. Detailed analysis has still to be done.

E.C.G.—They have not been analysed in detail though there are some interesting abnormalities.

14. Inquiry on clinical application of electro-retinography in cataract, glaucoma and detachment of retina under Dr. R.P. Dhanda at the M.G.M. Medical College, Indore.

1. To study the variations in electro-retinogram in different stages of cataract formation and after different methods of cataract extraction and thus to assess their prognostic value.

2. Similarly, to study various types of electro-retinogram in different types of glaucoma as well as in cases of detachment of retina and again to assess their prognostic value.

Electro-Retinography in cataract.—Twenty-two more cases of cataract were investigated during the year under report. Of these, 14 were mature cataract, 4 immature cataract, 1 hypermature cataract, 1 traumatic cataract, 1 congenital cataract, and 1 of an old aphakia. The average b-pot. of these cases was 0.225 mV as compared to 0.320 mV of normal Indian adult, as previously reported by the author. (Dhanda, *Annual Reports, 1952, 1954*; *Dhanda Arch. Ophth. Ame. Med. Assoc.*, **54**, No. 6; *Dhanda, Ind. Jour. Med. Res.*, **44** No. 2.) Of these, 14 cases were followed up and electro-retinogram repeated in some cases up to 9 months.

Investigation during the year further confirm the observation of last year that electrical response is only moderately sub-normal in cases of cataract even though the visual acuity has been reduced to hand movement and P.L. and P.R. This interesting point is a clear proof that even a faint stimulus passing through the opaque tissue is enough to stimulate retina to its activity provided, of course, functional capacity of retina is normal.

That electro-retinography is the most precise means of determining the prognosis of a particular case is proved by an extinguished electro-retinogram in an eye with mature cataract. The cataract was removed and a detachment of retina discovered. This explained the loss of electrical response (the other eye was normal with b-pot. of 0.30 mV.)

Post operative changes in electro-retinogram indicate fairly interesting observations as given below :—

Number of cases	Method of cataract extraction	Average b-pot. before operation	Average b-pot. 15 days after
5	Intra-capsular	0.260mV.	0.305mV.
5	Extra-capsular	0.267mV.	0.205mV.
1	Curette evacuation	0.30mV.	0.395mV.

In all the intra-capsular cases the average b-pot. after operation, increased in every case. In 5 extra, capsular cases, the b-pot. after operation decreased in 4 cases and was unchanged in 1. This observation possibly leads one to conclude that the retinal activity is disturbed more in cases of extra-capsular operation than in cases of intra-capsular operation. This incidently explains that the breaking of suspensory ligament in intra-capsular method does not pull on the retina, and, therefore, does not affect the function of retina. It may thus be presumed that the so-called increased frequency of detachment of retina, after intra-capsular surgery, may be incidental rather than factual. It

may be stated that the author having practised intra-capsular surgery for the last 13 years has yet to come across a single case of post-operative, detachment of retina in his cases.

Electro-retinography in glaucoma.—Seven eyes with chronic glaucoma were investigated. The average b-pot. was 0.171mV (the highest being 0.275mV, lowest being 0.05mV.)

Three eyes of secondary glaucoma had an average b-pot. of 0.233mV. (the highest being 0.40mV. and lowest being 0.1mV.)

One eye with absolute glaucoma had a b-pot. of 0.150 mV. with no P.L.

Number of eyes.	Type of glaucoma.	Average b-pot.
3	Congestive glaucoma. (Secondary to cataract).	0.233mV.
7	Chronic simple glaucoma.	0.171mV.
1	Absolute glaucoma	0.150mV.

These findings also throw interesting light on the pathology of glaucoma. The presence of fair amount b-pot., even in a case of glaucoma, with no preception of light, is strongly suggestive that the loss of vision in cases of glaucoma is due to its effect on the optic nerve only because the presence of electrical response from such an eye indicates that the functioning capacity of retina is present to a fair extent. This also incidently and possibly supports the hypothesis put forward by Dr. Mathur of Agra about the possible existence of a space called fourth chamber in front of optic disc. If that be presumed true, it could be explained that the effect of raised intra-ocular tension is transmitted to the optic disc through this space. Obviously, these observations prove that the retinal function continues to remain good even though it is exposed to a very high degree of intra-ocular tension. The higher b-pot. in cases of secondary glaucoma during the intumescence stage of cataract is explained by the short duration of pathology and also explains that the retinal activity being good, the return of vision after relief of tension in these secondary cases is expected to be good indicating good visual prognosis.

Electro-retinography in detachment of retina.—Five patients, each with detachment of retina in one eye, the other eye being normal were investigated. The electro-retinogram in most of the diseased eyes was either extinguished or showed very low electrical response (less than 0.10mV). Obviously, the prognosis in these cases was very poor and so surgery was not indicated. Detachment of retina is an uncommon condition in this country and most of the cases are seen in a late stage. It would be interesting to investigate the case in early stage when prognosis is good and study the change in electro-retinogram after operation.

CONCLUSIONS

1. Increase in b-pot. after intra-capsular operation, as compared to its decrease after extra-capsular operation, indicates less disturbance to the retina in intra-capsular surgery.

2. Electro-retinography is a very precise means of assessing the functional capacity of retina even though ophthalmoscopy is impossible.

3. A good electrical response in cases of chronic glaucoma, even with no perception of light, strongly suggests that pathological effect of glaucoma is mainly on the disc and around it. Retina, on the whole, retains good functioning capacity.

4. Electro-retinography is a very good mean of determining the prognosis in cases of detachment of retina.

15. Biochemical and serological investigations on *Escherichia* and *Klebsiella* strains isolated from urinary infections under Dr. S.P. Gupta and Dr. N.P. Gupta at the K.G. Medical College, Lucknow.

1. *Collection of strains.*—822 strains of coliform organisms, isolated from established cases of urinary infection, have been collected in this Department and are kept in sealed 1 per cent agar stabs.

2. *Biochemical investigations.*—So far 510 strains have been tested for their biochemical reaction with 27 media. The main differentiating tests between *Escherichia* and *Klebsiella* were: adonitol, inositol, indole, M.R., V.P., urease, KCN broth and their motility. *Escherichia* strains gave positive results with indole and M.R. and were motile, while *Klebsiella* gave positive results with adonitol, inositol, V.P., urease, KCN broth, and were non-motile. The strains could be divided on the above basis into 340 *Escherichia* and 162 *Klebsiella*. Eight strains could not be classified.

3. (a) Hæmagglutination tests were carried out on all the organisms. The results indicated that 106 strains, out of 510 tested, gave a positive hæmagglutination test. There was no correlation between the hæmagglutinating property and the serological type of *Escherichia* strains.

(b) Hæmolysis was tested with all the *Escherichia* strains and it was found that 46 possessed this property. There was no correlation between the hæmolytic activity and the hæmagglutinating property nor was it confined to any well-defined serological groups.

4. Serological studies of strains of *Escherichia* :

(a) *Determination of O group of Escherichia strains with the help of 25 O-sera (constituting the diagnostic scheme).*—An attempt was made to determine the O-group of these strains. After destruction of any surface antigens by heating, these organisms were subjected to slide agglutination with properly diluted O-sera. Of the 340 strains of *Escherichia*, 114 strains could be typed and were found to lie within the 25—O-groups of

the diagnostic scheme. Twenty-three strains belonged to O-group 20, 31 strains to O-group 15, 16 strains to O-group 6, and the rest were scattered in all the other 22 O-groups.

(b) *Determination of L-antigens of Escherichia strains.*—Sera against 12 antigens were prepared by injecting the freshly prepared antigens (growing the organisms on nutrient agar for 20 hours, testing their O-inagglutinability and then suspending in 0.85 per cent saline) intravenously in rabbits. Sera were preserved in 50 per cent neutral glycerine in sterile bottles.

The work of determination of L-antigens on *Escherichia* strains is in progress. Preliminary determination of L-antigens with 9 OL sera showed that the 310 strains tested with slide agglutination 144 strains could be typed.

5. Preparation of sera against 14 capsular antigens of *Klebsiella* (constituting the diagnostic scheme) is in hand.

16. Investigation into the role of allergens and various other factors in the production of bronchial asthma in Rajasthan in general and in Jaipur area in particular under Dr. R. M. Kasliwal at the S.M.S. Medical College, Jaipur.

The work was limited during the year 1956-57 to a study of the atmospheric and botanic flora in and around Jaipur city and evaluation of allergenicity of the pollens.

To accomplish this, a comprehensive survey of the botanic flora in and around Jaipur city was carried out as a preliminary measure. Various types of plant species growing here were determined and their relative abundance and distribution in different portions of this locality was ascertained. This study was carried out, in general, for three categories of plants, namely, trees, weeds, and grasses. Special emphasis was given to the study of anemophilous plants, which are reported to be of major importance in the causation of respiratory allergic disorders of pollen etiology. Pollinating periods and the mode of pollination were determined and a Pollination Calendar for the year 1955-56 was prepared.

Field collection of pollens was carried all round the year. Pollens were directly collected in the fields in glazed paper bags by plant-breeders method and also in Petri-dishes in case where tying of glazed paper bags was not possible. After collection, pollens were promptly dehydrated and defatted and stored in tightly stoppered containers in a refrigerator. Simultaneously, a microscopic study of pollens, so collected, was also done, first unstained and then after-staining with Calberla's solution. Drawings and photomicrographs of pollens and photographs of plant specimens were obtained for future reference. This study of known pollens was important to identify the aerial pollens subsequently.

A daily census of the pollens caught from the atmosphere at three different localities in Jaipur city was also done all round the year. This was done by the Gravity-slide method using a standard sampling device (Durham). The type and concentration of aerial pollens from a daily catch was recorded. Pollen counting was done by the cover-glass technique and expressed in terms of the number of pollen grains per sq. cm. of the slide area exposed in a horizontal position for a period of twenty-four hours (as recommended by the National Pollen Survey Committee of the American Academy of Allergy). Identification as to the individual species being difficult at this stage of study, differential pollen survey was carried out in general for three categories of plants, namely trees, weeds, and grasses.

A correlation was made between the aerial pollen incidence and pollination as determined by field survey of the local plant flora. A correlation was also made between the aerial pollen concentration and the incidence of respiratory allergy cases observed at S.M.S. Hospital, Jaipur.

In addition to the routine catch of aerial samples, slides were occasionally exposed at the patients residence in order to incriminate any local offending plant pollen.

Extracts of the dried and defatted pollens were prepared by extracting with glycerinated coca's solution at room temperature for 48 to 72 hours. Sterilization was done by bacterial filtration through Seitz filter and the sterility insured by sterility tests on Brewers thioglycollate medium. Standardization was done by weight volume (percentage system) method. As a routine, extracts with a strength of 1 in 50 were prepared and stored in a refrigerator to maintain the allergenic potency.

Allergenicity of the pollens was evaluated by diagnostic skin tests after a detailed clinical interrogation of the patient. Both the scratch and intracutaneous techniques were employed. Scratch testing was done, as a rule, routinely before I.C. testing. A few allergenic pollens were recognized.

Results.—The field survey revealed that in case of most of the trees the pollinating periods were from February to May. Majority of the grasses were found to pollinate all round the year, the maximum pollination was, however, found during the months of September to March. For most of the weeds the pollinating periods ranged between November and March.

Aerial pollen census showed that the maximum total pollen counts were found in the months of September, October and November. The maximum concentration of tree pollens was seen in the months of April, May and June. The concentration of pollens from grasses was found to be maximum in the months of September, October, and November just following the monsoon season. The pollen counts of weeds were found evenly distributed except for the two months of August and September, when it was found to be low, probably owing to the rains during these months. It was concluded that the maximum pollution of atmosphere in the environs of Jaipur city is from pollens of grasses.

These findings appeared to confirm the results of field survey.

Correlation of ærial pollen concentration and incidence of cases of respiratory allergy observed at S.M.S. Hospital, Jaipur, showed that the later more or less paralleled the ærial pollen fluctuations.

17. Inquiry on suprarenal activity in experimental cholera under Dr. S. N. De at the Medical College, Calcutta.

Diarrhœa has been produced in 60 per cent of adult rabbits by intra-intestinal injection of culture of *Vibrio cholerae* with mucin after ligation of part of the huge cæcum of these animals. There was evidence of slight hæmoconcentration (rise of packed cell volume, erythrocyte count and hæmoglobin percentage) and of fall of blood pressure followed by death of some of the animals after the second day. Hæmoconcentration could not account for the death of the animals. *V. cholerae* was recovered on culture of rectal swab, liver and spleen, but not from heart's blood. Changes in leucocyte count were less marked than in the controls and it could not give an idea of suprarenal activity in the test animals.

In animals pretreated with injections of cortone for three days, the same experimental procedure was followed by death of all the animals and diarrhœa in all but a few which died too early. Similar changes in blood pressure and blood picture were observed. Suprarenal activity could not be assessed from leucocytic changes. Pre-treatment with cortone seems to depress suprarenal function and predispose the animals to experimental cholera and to earlier death therefrom.

18. Enquiry on electrophoretic studies on normal and pathological human sera under Dr. N. C. Datta at the Grant Medical College, Bombay.

It was found after a number of trials with various buffer mixtures and different staining technique, that the method described by Jencks, Jetton and Durum (1955) gives most satisfactory results and a good protein pattern.

1. Investigation carried out.

(a) To set up normal values from blood of healthy volunteer.

(b) Normal Pregnancy.

(c) Aneamic pregnant women and those who show signs of toxæmia or eclampsia.

(d) Infective conditions like Diphtheria, Typhoid etc.

2. *Liver diseases.*—A study of 26 cases of cirrhosis of the liver the characteristic albumin depression and elevation of gamma globulin. In billirary cirrhosis, there is also a less marked decrease in albumin and a increase in gamma globulin, in addition, in certain cases there is

an increase of Beta globulin. Similar increase has also been reported by others. The extent of liver damage in chronic protein malnutrition is not indicated by determination of albumin and globulin value but a definite elevation of gamma globulin by paper electrophoresis.

19. Enquiry on the formation, distribution, structure and development of the splanchnic nerves under Dr. Inder Jit at the Medical College, Amritsar.

The origin, formation, distribution and termination of all the three splanchnic nerves have been followed in 35 adult human cadavers (70 sides) and in one full term foetus. Histological structure was examined by making serial sections in the case of one full term foetus and making complete serial sections of 2 embryos.

In 80 per cent cases the sympathetic trunk had 11 ganglia. In 73 per cent cases stellate ganglion was present. Thoracic cardiac nerves were found taking origin from first thoracic sympathetic ganglion or stellate ganglion and 2nd ganglion and not below that level. Lobstein's ganglion was found in 27 instances. Para-aortic nerve was present in 11 instances in left side and one instance in right side. Nerve of Swan was present in 8 instances. Collateral ganglionated trunk of Kondratjew was found in 4 instances. Another nerve was found which simulated highest splanchnic nerve described by Wrisberg.

20. Inquiry on the value of commercial silk grafts and homografts in bridging large blood vessel gaps under Dr. Yudhveer Sachdev at the Medical College, Amritsar.

The preliminary work of the study and comparative evaluation of suture methods of vascular anastomosis was completed last year. Along with this the use of intravenous nembutal as an anæsthetic was studied. The transplantation of aortic grafts was undertaken after completion of the preliminary study of various suture methods. In the first group of dogs fresh homologous aortic grafts were used and in the second group aortic grafts preserved in 4 per cent neutral formal saline were used.

MATERIAL AND METHODS

Transplantation of homologous aortic grafts has so far been done on 15 dogs. In all fresh homologous aortic grafts taken from the abdominal aorta were transplanted in the abdominal aorta below the origin of renal arteries. Two dogs of about equal size were selected and a piece of abdominal aorta varying from 2 cm. to 3.5 cm. was removed from one (donor dog) using aseptic technique. Excessive periarterial tissue was removed and branches in this piece were ligated. This piece was kept in sterile normal saline, while the second (recipient dog) was being operated. In the second dog an appropriate piece of the aorta was excised between two aortic clamps applied after complete mobilization of the aorta below the renal arteries. Then the graft already removed from the donor dog was transplanted using continuous mattress suture for anastomosis. The

clamps were removed, first distal and then proximal, any blood leakage was stopped with slight pressure or one or two extra sutures. These experiments were done under intravenous nembutal sodium (2.5 per cent) anaesthesia. Proper aseptic precautions were taken. No antibiotics or anticoagulants were used.

In the second group, aortic homografts preserved in 4 per cent neutral formal saline were transplanted. Neutral formal saline was prepared by making a 4 per cent solution of formaline in normal saline and neutralizing it by adding excess of calcium carbonate. So far, 4 formal saline-preserved grafts (11, 14, 14, 17 days) have been transplanted. More such grafts will be used in due course.

RESULTS

1. Out of the 12 dogs in the first group, 2 died ; one after 4 days and the other after 7 days. Post mortem was done in the 6 dogs after 1, 2, 7, 10, 12 and 14 days. Four dogs are still under observation after 89, 86, 56 and 32 days, respectively.

2. Out of 4 formal saline-preserved grafts, one dog died after 4 days due to dehiscence of anastomosis and the second dog of pneumonia after 11 days. Two dogs are still under observation (26 and 3 days)

21. Study of renal changes following ureteric ligation and estimation of recovery following release of obstruction by ureteric transplantation under Dr. B. N. Balakrishna Rao at the G. R. Medical College, Gwalior.

The object of this inquiry (*vide* preliminary report of last year) was the determination of the critical time period for reversal of hydronephrotic changes. In furtherance of the above object, the work of the previous year was followed in the Department of Experimental Surgery, Gajra Raja Medical College, Gwalior. Besides the determination of critical time period, work on other aspects of experimental hydronephrosis was also undertaken. (*vide infra*).

It had been an established belief of general practitioners/clinicians and urologists that sudden complete obstruction to the outflow of urine leads to a primary atrophy of the kidney which rapidly reduced to a flattened fibrosed mass. This idea is also propagated in most books on urology and surgery. The present work proves beyond doubt that sudden complete obstruction invariably produces hydronephrosis and this point should no longer be now seriously contested. In not a single instance primary atrophy of kidney following surgical ligation of ureter has been observed. The gross and microscopic changes were described in detail in the last report (1955) of this inquiry.

Current work on other interesting aspects of experimental hydronephrosis.—The elucidation of following aspects of experimental

hydro-nephrosis is being carried in the Department of Experimental Surgery, Gajra Raja Medical College, Gwalior.—

- (i) Suitable means and methods are being devised and tested for experimental production of hydronephrosis by intermittent obstruction. It is proposed to contrast the results obtained with hydronephrosis that was produced by sudden complete and continuous obstruction. To determine the critical time period (if at all it differs from hydronephrosis produced by sudden complete obstruction) for hydronephrosis produced by intermittent obstruction.
- (ii) Factors concerning the etiology of so-called idiopathic hydronephrosis (hormonal and autonomic nervous system disorders), are also being studied. The effect of coeliac ganglionectomy in production of idiopathic hydronephrosis are being tried. It is also contemplated to find out the effect of nerve stripping pelviureteric junction in production of hydronephrosis. To work out the difference of idiopathic hydronephrosis (if successful) with that of obstructive variety.
- (iii) To visualise the lymphatics of normal and hydronephrotic kidney by injection, at suitable sites of 1 c.c. of 11 per cent Patent blue. This is being done to elucidate (if any) the role of lymphatics in absorption of hydronephrotic sac contents.
- (iv) The technique of obtaining electrophoretograms have been standardized and work on electrophoresis of serum and hydronephrotic sac contents is being done.

22. Inquiry into experimental production of pneumoconiosis and emphysema under Dr. R.K. Goyal at the S.M.S. Medical College, Jaipur.

As the important constituents of Jaipur dust are silica and silicates, injections of Jaipur dust were given by different routes in different species of laboratory animals (rabbits, guinea-pigs, rats, mice and monkeys), a number of other foreign particles and chemicals were injected as controls, e.g. Coonoor dust, silicon-di-oxide, quartz, feldspar, animal charcoal, etc. The results are as follows :—

Rabbits.—Rabbits were found on the whole, to be extremely susceptible to the injection of silicon-di-oxide, even 1/20 mg. injected intravenously was lethal in about four weeks time, bigger doses caused an earlier fatality. Most of the rabbits injected with silicon-di-oxide by different routes (intra pulmonary, intra-tracheal, intra-peritoneal) developed intercurrent pulmonary infection and died at varying periods depending on the dose, the minimum period being 24 hours. The lesions caused were necrosis and thrombosis of vessels. Only a small minority of rabbits resisted gradually increasing doses of silicon-di-oxide injected by the intratracheal or intraperitoneal route and developed granulomatous lesions in the lungs. In a number of preliminary experiments when

silicon-di-oxide was coated with ferric phosphate or aluminium hydroxide, the noxious effect of silica was minimised.

The intra-pulmonary injection of 6.0 mg. of Jaipur dust was lethal in 8 to 13 days time, the injection of 10 mg. to 20 mg. of the same dust by the intraperitoneal route also proved fatal in 4 to 11 days time. The intra-pulmonary injection of 3.0 mg. of Jaipur dust was tolerated by most of the rabbits, but after receiving a number of injections at fortnightly intervals most of the rabbits developed bronchopneumonia, granulomatous lesions were produced only occasionally.

Coonoor dust proved to be more toxic than Jaipur dust ; even the intrapulmonary injection of one mg. given repeatedly proved toxic. Similarly, the injection of quartz or Kieselgurh in 1.0 mg. doses given repeated proved toxic.

Much bigger doses could be administered subcutaneously but no definite local granulomatous lesions were produced, even 20 mg. were tolerated by this route. The intrapulmonary injection of animal charcoal was given as a control. the injections given by this route make the rabbits extremely susceptible to intercurrent infection and they develop snuffles which is generally followed by pulmonary infection. The intratracheal injections have been started and the preliminary experiments carried out indicated that this is a more satisfactory route of injection, gradually increasing small doses of silicon-di-oxide given at 3 to 4 weeks interval appear to produce tolerance to the noxious agent. More experiments have to be carried out along these lines. The rabbits have not proved to be very satisfactory animals for production of pneumoconiosis due to the marked susceptibility to pulmonary infection.

Guinea-pigs.—Whereas an injection of 1/20th mg. of silicon-di-oxide was lethal to rabbits, the average lethal dose of silicon-di-oxide for guinea-pigs administered intraperitoneally was 300 mg. An intra-peritoneal injection of 750 mg. of Jaipur dust was tolerated by a guinea-pig. A single injection of 150 mg. to 200 mg. of silicon-di-oxide administered intraperitoneally did not produce granulomatous lesions or marked interstitial pneumonitis. The preliminary experiments indicate that repeated injections given intraperitoneally or by the intrapulmonary route at 2 to 4 week intervals are suitable for production of interstitial pneumonitis, granulomatous lesions and emphysema. The injections of Kieselgurh and black mica were not satisfactory. The administration of feldspar gave variable results in a small series of experiments. Repeated injections of dust from Coonoor also produced granulomatous lesions in a number of cases. The minimum period for the production of definite lesions appear to be more than three months, a period of 8 to 10 months is more likely to produce definite lesions. The subcutaneous route was found to be unsatisfactory for the production of pulmonary lesions.

Rats.—The rats are highly resistant to the action of silicon-di-oxide. The average lethal dose of silicon-di-oxide for rats weighing 150 g. to 200 m.g. Big single doses of silicon-di-oxide administered intratracheally or intraperitoneally are unsatisfactory for the production

of granulomatous lesions and interstitial pneumonitis. The preliminary experiments show that gradually increasing doses of quartz coated with ferric phosphate (which is similar in composition to Jaipur dust) administered at 2 to 4 weeks intervals can produce marked granulomatous lesions in more than six months time.

Mice.—The lethal dose of silicon-di-oxide for mice weighing 25 g. to 30 g. is 20 mg., whereas the lethal dose of Jaipur dust is 40 mg. Administration of 20 mg. of Jaipur dust by the intraperitoneal route was unsatisfactory, whereas 30 mg. of the dust produced marked interstitial pneumonitis in about one month in one case. When a single dose of 30 mg. of Jaipur dust given intraperitoneally was followed by a second dose of 15 mg. after a month, the mouse died within 48 hours. This observation suggests the existence of either a cumulative effect or a drug allergy.

Monkeys.—The monkeys were found to be highly resistant to the intraperitoneal injection of 1,000 mg. to 2,000 mg. of silicon-di-oxide, the intratracheal injection of 1,000 mg. to 2,000 mg. was also tolerated. These single large doses failed to produce any definite lesions in the lungs. A dose of 250 mg. of silicon-di-oxide was found to be lethal on intravenous injection, whereas 100 mg. injected intravenously was tolerated. When 100 mg. of silicon-di-oxide were re-injected, intravenously, on the 10th day the monkey died within 2 to 3 minutes. This observation suggests the existence of either a cumulative effect or drug allergy.

Silica content.—The silica content of normal lungs in the West is 0.25 per cent it may go up to 1.0 per cent without producing definite lesions. the silica content of apparently normal lungs in Rajasthan varied from 1.5 per cent to 4.6 per cent in a small series of experiments and in a few animals injected with silicon-di-oxide or Jaipur dust, it ranged between 3.5 per cent and 12.5 per cent.

23. Investigations in the use of certain Ayurvedic preparation in the treatment of *Diabetes mellitus* under Dr. R. V. Sathe at the J. J. Group of Hospitals and the Grant Medical College, Bombay.

The investigations in the use of 'Jasad Bhasma' (zinc) in Ayurvedic form were being done in the J. J. Hospital for the last two years. The procedure of investigation adopted has been detailed in the last year's report. Seven cases were reported last year. Much of the clinical material investigated this year has been wasted. Out of the 120 cases investigated (except 20 cases which are under report, 100 cases came irregularly and so could not be followed up.

Results of treatment with 'Jasad Bhasma'.

(1) *Urine volume.*—The 24-hour urine excretion which was above normal came to within normal limits.

(2) *Urine sugar and blood sugar.*—The 24-hour urine sugar excretion before treatment varied from trace to 218 gs. At the time of discharge in eleven cases, under treatment with zinc, there was no sugar in 24-hour urine sample. 1 g. to 10 g. in 4 cases and between 10 g. to 25 g. in two cases : and two cases passed more than 25 g. of sugar a day.

The fasting blood sugar of all cases was considerably high on admission. It varied from 160 mg. per cent. to 369 mg. per cent. At the time of discharge these blood sugar values were considerably lowered. Before start of the treatment there was no case having fasting blood sugar below 120 mg. per cent one case below 160 mg. per cent (mild diabetes), seven between 161 mg. and 200 mg. per cent (moderately severe) and above 201 mg. per cent (severe diabetes) there were eleven cases. After treatment with 'Jasad Bhasma' nine cases showed fasting blood sugar below 120 mg. per cent six between 121 mg. and 160 mg. per cent (i.e. mild diabetes), three remained moderately severe and only one case remained as severe diabetes, i.e. the fasting level was above 201 mg. per cent. Blood sugar levels in three cases did not show marked alteration in their blood sugar as a result of treatment due to complications, such as tuberculosis, hepatopathy and long-term uncontrolled diabetes.

The blood urea and blood cholesterol levels were found to be within normal range throughout the period of observation in all cases except one. Four cases who had already high blood cholesterol before treatment came to normal level after treatment. The other findings are just like those of last year.

Liver function tests were found to be normal in 18 cases and in two cases there was some deviation before treatment. No noticeable deviation occurred in any case during or after treatment.

Result of zinc 'Jasad Bhasma' treatments are divided into three categories.

The patients were divided into three age groups: (a) Above 56 years (b) between 41 and 55, and (c) below 40 years. Those who reacted (a) satisfactorily were in group a—one, in group b—five, in group c—four (b) moderately satisfactory were in group a—one, in group b—three and in group c—nil. (c) Unsatisfactory in group a—nil, in group b—three and in group c—two.

One case in group b was kept on diet only hence it has not been included in the above list. Group A had 2 cases with complications, one had retinopathy and the other had pleurisy. Of these the case with retinopathy improved very well and even his vision which was 6/12 to 6/18 before treatment improved to 5/6 to 6/8 and the retinopathy also improved.

In group b, out of the 11 cases, 3 were kept on diabetic diet only for 15 days with placebo but there was no improvement in urine sugar excretion or blood sugar. These cases, however, improved with zinc. One case in this group was resistant to insulin, on basal diet he passed 10 g. of sugar per day in urine and his blood sugar fasting was 268 mg. per cent, with diabetic diet for 17 days he passed 126 g. percent of sugar.

So, he was put on insulin which was raised from 20 to 100 units in 20 days but he still passed 4 g. of sugar. This showed that there might be some anti-insulinogenic factors, insulinase or alike which might be neutralizing the insulin injected. After zinc tablets were started, he steadily improved and his fasting blood sugar came from 268 to 122 mg. per cent.

Insulin and its relation to zinc treatment.—Fifteen patients were taking insulin before treatment and five had not taken insulin. The dose varied from 20 to 104 units. Out of the 19 cases under zinc, two required insulin and the sugar excretion after zinc treatment without insulin was as under :—

Sugar excretion nil in 24 hours.....	11 cases
„ „ trace-10 gms.....	3 cases
„ „ 11 g. to 20 g.....	2 cases

Four cases were treated with zinc only and no insulin.

Relation of diet to sugar excretion.—

Total number of cases on diabetic diet.....	14
(a) Reduction in sugar in 24 hours urine.....	5
(b) No change in urine sugar.....	3
(c) Increase in urine sugar.....	4

Fasting blood sugar changes in 2 cases which were kept on diabetic diet for 2 weeks were as under .—

Case No. 1 From 220 mg. per cent to 200 mg. per cent.

Case No. 2 From 170 mg. per cent to 160 mg. per cent.

Diet has not shown any appreciable change in blood sugar level of these cases.

Relation of treatment to weight.—Weight was reduced in 7 cases, stationary in 3 and increased in 11 cases. The reduction was from 1 lb. to 10 lb. and increase from 2 lb. to 22 lbs.

Relation of zinc treatment in the recovery of diabetic state.—It will be seen that in the selected group of diabetic cases only one case was mild, seven were moderately severe and 12 severe, of these only 6 cases were without complication and 14 had complications, such as tuberculosis, diabetic retinopathy, hepatopathy, neuropathy and hepatitis. Taking these facts into consideration 57·8 per cent showed satisfactory results 15·7 per cent were moderately satisfactory and only 26·5 per cent did not react satisfactorily. The percentage of complicated to uncomplicated diabetic case was 70 per cent to 30 per cent.

The time relation for the effect of zinc to be seen on the diabetic syndrome varied with individual cases, taking into consideration the security and complication and also the age group of the patients. Those with maturity onset type of diabetes with recent onset and no complication react vary early within 2 to 3 months, while those with severe diabetes and tuberculosis reacted after 6 months. All these findings

show that zinc in the form of *Jasad Bhasma* has a definite place in the treatment of *Diabetes mellitus*. It only remains to be seen how zinc works and with that aim in view it is proposed to undertake investigations.

24. Inquiry on the metabolic fate of gelatin administered as a plasma substitute under Dr. M. Damodaran at the National Chemical Laboratory, Poona.

The object of the investigation is to study the metabolic fate of transfusion gelatin prepared in this Laboratory with a view to developing this material into an ideal plasma substitute.

Work on the inquiry was commenced from the middle of March 1956 with the appointment of an Assistant Research Officer.

The estimation of molecular weights by osmotic pressure and light scattering methods was standardized and the weight average molecular weights of gelatin samples treated with trypsin for varying periods were determined. Animal experiments were started with the gelatin samples. Experiments were carried out to study the effect on haemo-dilution, survival rate and histological pattern of the internal organs produced by the intravenous administration of 8 per cent gelatin solution of known molecular weight. This part of the study has now been completed and the results can be summarized as follows :—

1. Intravenous administration of an 8 per cent gelatin solution to adult albino rats at a level of 15 c.c. kg. body-weight resulted in a slight increase in the erythrocyte sedimentation rate and coagulation time and a significant reduction in the concentration of red cells, haemoglobin and plasma protein and in w.b.c. count and haematocrit at 2 and 24 hours after transfusion. When gelatin was administered at 24 hour intervals for a period of 3 days, a cumulative effect on haemo-dilution was observed. Significant alterations in the blood picture were not, however, apparent when gelatin transfusions were given twice a week for a period of two weeks.

2. The technique and conditions for the production of experimental shock in rats were standardized. A study on rats bled to nearly half the original blood volume from the heart or jugular vein under nembutol anæsthesia and infused with an equal volume of 8 per cent gelatin solution has shown that gelatin has good shock-combating effect, the animals showing a good prognosis of recovery from the experimental shock and a high survival rate. The animals showed no reaction during or after transfusion.

3. The internal organs, viz. heart, liver, kidney and spleen removed from rats receiving 2 c.c. of 8 per cent gelatin per week intravenously for a period of 3 weeks and from animals bled to nearly 40 per cent of the original blood volume and sacrificed 48 hours after infusion with an equal volume of gelatin did not show accumulation of gelatinous material in the tissues or any morphological changes microscopically. Detailed histological

examination of these internal organs is now being carried out in collaboration with the Pathology Department of the Armed Forces Medical College, Poona.

The metabolic behaviour of transfusion gelatin in relation to molecular size was taken up next for investigation. The studies now in progress have been directed towards the following aspects :—

1. The rate of excretion and percentage retention in blood in relation to the molecular weight distribution of the transfused gelatin.
2. The effect of transfusion gelatin with and without supplements of tryptophane and cysteine on maintenance of nitrogen balance and formation of hæmoglobin and plasma protein.

In these studies doubly depleted dogs and rats maintained on low-protein diets are being used.

25. Study of distribution of atherosclerotic heart disease in various social groups in Delhi and its correlation with serum cholesterol and lipid studies under Dr. (Km.) S. Padmavati at the Lady Hardinge Medical College, New Delhi.

CLINICAL MATERIAL USED

1. Patients.

(a) Sixty-eight cases of hypertension and seven cases of atherosclerotic heart disease have been fully studied.

(b) A special group of patients suffering from atherosclerotic heart disease before the age of 40 years is also being studied.

2. Normal cases.

(a) *Industrial workers*.—486 male labourers from the Delhi Cloth Mills forming 5% of the total population of these mills have been examined. Their ages ranged from 18 to 59 years and 80·5 per cent fell into the age group 20 to 40 years.

(b) *Rural population* as represented by the patients attending the Najafgarh Primary Health Centre at Najafgarh are also being studied. 422 cases (both men and women) have been examined so far.

(c) *Small social and occupational groups* of not less than 25 each are also being studied. They were chosen either on the basis of having the same income (and perhaps the same dietary habits) or of following the same occupation or because they lived on a special type of diet. Examples are :—

(i) Coolies—males aged 16 to 60 years working in the Lady Hardinge Hospital.

(ii) Gardeners—male gardeners (malis) aged 20 to 65 years.

(iii) Medical students—female students aged 19 to 25 years.

The number of people being studied in the groups mentioned below has not reached any considerable figure as yet.

(i) Electricians.

(ii) Building coolies engaged in hewing stones and other heavy manual labour.

(iii) Nurses.

(iv) Doctors in general practice.

(v) Businessmen, mostly executives.

(vi) Lawyers.

(vii) Clerical workers.

(viii) Communal groups who live on a special type of diet, such as Sikhs and Kashmiris, eating a high-fat and high-meat diet, Marwaris on a high-fat vegetarian diet, etc.

METHODS OF INVESTIGATION

1. *History*.—The two groups, *viz.*, patients with heart disease and normal cases were interrogated on two separate types of proformas printed especially for the purpose.

2. *Dietary history*.—As there is no nutritionist in the team, at present, the dietary history is taken purely by interrogation. Stress has been laid on the total caloric value of the diet and its fat and cholesterol content.

3. *Physical examination*.—In this investigation all types of arteriosclerosis have been included in order to make the report comprehensive. The following criteria were used in the diagnosis of atherosclerosis and hypertension.

A. Arteriosclerosis.

(a) Coronary atherosclerosis—

(i) History of myocardial infarction or angina pectoris with or without electro-cardiographic evidence.

(ii) Purely electrocardiographic evidence of coronary artery disease, such as T-wave changes, *a-v.* block, bundle branch block, etc., when these could not be attributed to other causes.

(b) Retinal arteriosclerosis as seen on fundoscopy.

(c) Thickened and pulsating peripheral arteries, such as brachial and radial.

(d) Radiological evidence, such as uncoiling of the aorta.

True atherosclerosis for the purpose of this enquiry was only coronary atherosclerosis.

B. Hypertension.

Systolic and diastolic blood pressures over 140 mm. Hg. and 90 mm. Hg., respectively, recorded on more than one examination. Elevation of only the systolic blood pressure was designated as systolic hypertension.

All the patients as well as the normal cases had a complete physical examination and their height and weight recorded. An attempt is also being made to study the somatotypes in these various groups.

4. *Biochemical tests.*—The blood lipids estimated were :

(i) Total and free cholesterol by the iron-reagent method of B. Zak *et al* (1954).

(ii) Ester cholesterol was evaluated from the above values.

(iii) Phospholipids of serum were estimated as lecithin by the method of Zilversmit and Davis (1950).

(iv) In a few cases blood urea and uric acid estimations were also done. The blood samples in all these cases were random specimens collected after calling the men from their work.

5. *Electrocardiography and fluoroscopy.*—These were done wherever facilities permitted. These have been done in all the small occupational groups mentioned and in a number of the rural and industrial workers.

RESULTS OBTAINED

1. Patients.

A. The sixty-eight cases of hypertension were classified as follows :—

<i>Etiology</i>	<i>Number of cases.</i>
(i) Benign essential hypertension	28
(ii) Malignant hypertension	3
(iii) Diabetes with hypertension	4
(iv) Other causes such as renal disease and toxæmia of pregnancy, etc.	33

The blood lipid levels in this group were not significant.

B. The number of young atherosclerotics studied up-to-date is too small for any conclusions to be drawn about them.

2. Normal cases.

The results in these cases have been classified under the following three heads :—

- (i) Incidence of hypertension and atherosclerotic heart disease.
- (ii) Dietary fat and cholesterol.
- (iii) Blood lipid levels.
- (i) & (ii) The incidence of hypertension and coronary atherosclerosis,

and the dietary fat and cholesterol intakes in the various groups are as follows :—

Group	Number of cases	Incidence of hypertension, per cent	Incidence of coronary atherosclerosis, per cent	Average daily fat intake in g. (range in brackets)	Average cholesterol intake in mg. (range in brackets)
1	2	3	4	5	6
1. Industrial workers from the Delhi Cloth Mills (males)	486	1.2	Nil	68 (0—225)	200 (0—800)
2. Najafgarh population (males and females)	422	1.13	Nil	50 (0—250)	132 (0—640)
3. Hospital coolies (males)	27	Nil	0.38%	52 (0—205)	168 (0—684)
4. Gardeners (males)	22	Nil	Nil	23 (0—83)	69 (0—234)
5. Medical Students (females)					
(i) Vegetarians	7	Nil	Nil	93 (30—96)	175 (108—254)
(ii) Non-vegetarians	18	Nil	Nil	89 (66—122)	405 (89—611)

In this survey fats contributed to 8.3 to 27.9 per cent of the total calories consumed. In a few cases the amount of fat consumed was enormous viz., 250 g. per day. These figures need further checking by proper weighing of the diets.

(iii) *Blood lipid levels.*—An attempt was made to correlate the blood lipid levels in each group with the calculated fat intake, the cholesterol intake and the age. In this survey no correlation of serum lipid levels was found either with the dietary fat, and cholesterol or with age in any of the groups. The average serum lipid levels of the various groups are shown in the following diagram. For the sake of convenience they have been grouped as above and below the age of 40 years. As is evident from this diagram the serum lipid levels in this survey were similar to those reported by others.

26. Enquiry on the role of *Ps. Pyocyaneus* in infections of women and children under Dr. S.L. Bhatia at the Lady Hardinge Medical College, New Delhi.

Strains of pseudomonads were collected from routine specimens sent to the laboratory. Sources of strains are detailed below. Only those cases were included in which *Ps. pyocyaneus* was grown in pure culture or was the predominant organism or grew in association with organisms of doubtful pathogenicity.

Organisms were selected from plates primarily on the basis of colour production, characteristic odour and colonial appearance. In the case of mixed cultures purity was assured by plating on desoxycholate—citrate agar and selecting single colonies. The specimens consisted of stools, urines, ear swabs, eye swabs, cervical swabs, pus and swabs from burns.

The incidence of *Ps. pyocyaneus* in these specimens were as follows :—

Stools	cultured	...	438		
	<i>Ps. pyocyaneus</i> pure culture	4	...	0.9%	
	„ „ predominant	12	...	2.7%	
	„ „ with other organisms	55	...	12.6%	
Urine	cultured	...	945		
	<i>Ps. pyocyaneus</i> pure culture	30	...	3%	
	„ „ with other organisms	90	...	9.5%	
Throat swabs	cultured	...	2858		
	<i>Ps. pyocyaneus</i> grown	24	...	0.8%	
Cervical swabs	cultured	...	2348		
	<i>Ps. pyocyaneus</i> pure culture	60	...	2.6%	
	„ „ with other organisms	20	...	0.9%	
Pus	cultured	...	320		
	<i>Ps. pyocyaneus</i> grown	32	...	10%	
Burn swabs	cultured	...	16		
	<i>Ps. pyocyaneus</i> grown	10	...	62.5%	
Eye smears	cultured	...	769		
	<i>Ps. pyocyaneus</i> grown	33	...	4%	
Ear swabs	cultured	...	440		
	<i>Ps. pyocyaneus</i> grown	210	...	48%	

From the lists given above it will be seen that the percentage of isolations in the case of ear swabs (from children with otitis media)

was very high. It was decided, therefore, to investigate the ear swabs of children with no history and no evidence of ear infection. One hundred such swabs were collected from the Out-patient's of the Kalawati Saran Children's Hospital. Moist fine swabs were used and these were cultured within 30 minutes of taking on to Difco agar plates. The plates were examined after 24 hours and 48 hours incubation at 37°C. Out of 100 cases 4 cases showed a scanty growth of *Ps. pyocyaneus*.

Antibiotic tests were carried out on 150 strains. These strains were selected only if the organism was the sole or the predominant organism. Only typical *Ps. pyocyaneus* strains were selected and the source of the specimens was as follows :—

Stools.	11
Urines.	27
Throat swabs.	3
Cervical swabs.	14
Pus.	7
Burns swabs.	7
Eye smears.	10
Ear swabs.	71

Filter paper disc technique was used and the following results were obtained.

	Definitely sensitive	Slightly sensitive	Sensitive resistant colonies	Resistant
Penicillin.	Nil	Nil	Nil	150
Streptomycin.	86	10	12	42
Aureomycin.	Nil	Nil	Nil	150
Terramycin.	16	6	18	110
Chloramphenicol.	22	13	8	97
Polymyxin.	130	20	Nil	Nil

According to Bushby 1955 diffusion technique is not to be recommended for polymyxin sensitivity tests. Fairbrother and Jennings 1955, however, state that filter paper technique is satisfactory for testing the activity of polymyxin against a wide range of common pathogens. In our tests with polymyxin the results were clear cut and very few resistant colonies were encountered. The maximum zone of inhibition was smaller than with other antibiotics. All strains of *Ps. pyocyaneus* were sensitive to polymyxin and this in agreement with the work of Wright 1954. This author states that all true *pyocyaneus* strains are sensitive to polymyxin and in fact suggests a classification of the group according to their sensitivity to this antibiotic.

Strains of *Ps. pyocyaneus* from pathological material which grew in pure culture on primary inoculation, were selected for typing. These strains are being maintained for this purpose.

O sera were prepared from rabbits by the technique used by Taylor for preparation of sera against coli strains. The organisms were grown in broth overnight—the suspension streamed for $2\frac{1}{2}$ hours and tested for sterility. Intravenous injections of the suspension were given twice weekly, the volume of successive inoculation being 0.25 ml, 0.25 ml, 0.5 ml, 0.5 ml, 1 ml, 1 ml, 10.5 ml, 1.5 ml. Test bleeding from heart blood was taken 4 days after the last injection. The sera collected has tested for agglutination by slide and tube technique Fox and Lowbury 1953. In all cases 2 more injections 1.5 ml. were required. No attempt was made to prepare sera in hot weather as we had learnt from past experience that titres in summer were low and often the animal died of heat or intercurrent infection.

Three satisfactory O sera are now ready and we are in communication with Christie and have asked for his standard strains and sera for comparison with our own. Others are being prepared. An attempt was made to carry out haemagglutination tests according to the method used by Neter & Walker 1954. The medium recommended by these authors was not available in India and glucose Difco agar plates were used. The test was not successful in the *pyocyaneus* strains and also in strains of coli obtained from Dr. Taylor using standard sera from Colindale. The medium required has now been ordered and we plan to carry out the tests as soon as it is available.

An attempt was made to type the strains of *pyocyaneus* organisms isolated from various pathological lesions with the 3 sera which were already prepared. The results are set out as follow :—

Total number investigated	...	137
Group I	...	69
Group II	...	2
Group III	...	27
Rough strains (auto-agglutinable)	...	13
Strains not agglutinated by Sera I, II, and III		26

Among the strains isolated, two of them were obtained from Blood cultures sent for culture of *Salmonellae* organisms. In both cases the sera of the respective patient agglutinated the organism from the patient.

27. Study of the role of immunologic mechanisms in the production of aplastic anæmia under Dr. V. S. Mangalik at the K. G. Medical College, Lucknow.

The purpose of the present investigation was to induce hypoplastic changes in the erythroid elements of the bone marrow after administration of anti-erythrocytic serum and to study the mechanism of the change so induced in the bone marrow.

TECHNIQUE

Animals.—Adult guinea-pigs of mixed strain, weighing from 350 g. to 400 g., were employed in this study. Some animals were splenectomized. The splenectomized animals were used after 15 to 20 days of the operation.

Anti-erythrocytic serum was prepared by injecting guinea-pig red cells into rabbits.

Injections.—Varying amounts of immune serum was injected by different routes. Sixty-four animals were employed in this study.

HAEMATOLOGIC TECHNIQUES

Hæmatologic studies were made on alternate days in every group on blood obtained from ear veins. At least 1,000 red cells were counted in each instance. Standard hæmatologic techniques were employed. Price Jones curves were constructed to determine the mean red cell diameter. Mean red cell thickness and Spherical Index were calculated. For the determinations of corpuscular constants and fragility of red cells, a separate group of 16 guinea-pigs was employed. Of these, 8 animals were splenectomized. Formation of this group was undertaken because of the necessity of removing at least 2.0 c.c. of blood for the above determinations.

Bone marrow aspiration.—At the height of changes in peripheral blood, bone-marrow studies were made. Blood samples were also obtained for demonstration of hæmagglutination.

Urine examination.—Hæmoglobinuria could be detected by naked eye examination and was confirmed by benzidine test. Urobilinogen was qualitatively determined by Schlesinger's test.

RESULTS

1. *Effect of normal guinea-pig serum.*—Little variations were noted in the number of erythrocytes and the value of hæmoglobin in animals administered with normal rabbit serum for a period of 15 days. The reticulocytes ranged between 0.25 and 1.0 per cent. Peripheral blood-smear examination revealed in all instances, well hæmoglobinized red cells, of uniform size and shape.

2. *Effect of intra-cardiac injections.*—In 5 animals the immune serum was injected by the intra-cardiac route. Profound anæmia was observed to develop in all the animals immediately following the injection of the serum. By the second day, the red cell number had fallen from the pre-injection level of 5.2 millions to 2.75 millions per cu. mm. Examination of the blood smears showed that the red cells were uniformly small in size and there was a marked degree of leucocytosis. Bone marrow activity as judged by peripheral reticulocytosis was completely absent. In one animal reticulocytosis was, however, observed as a terminal phenomenon. Examination of the bone-marrow smears at the height of the changes in the peripheral blood showed marked hypocellularity.

As early as 4 hours after the injection, animals were observed to pass urine coloured with blood; and by 24 hours uribilinogen was detectible in urine in large amounts. The anæmia was fulminant in its character and its development was so rapid, indeed, that by the 4th day all animals in the group had died.

At autopsy, the peritoneal cavity was found to contain hæmorrhagic exudate. The lung, liver and spleen were of normal size and showed the presence of varying numbers of hæmorrhagic spots on their external surface. Similar hæmorrhagic areas were seen on the pericardium as well. Microscopically, both the liver and spleen showed evidence of intense congestion.

3. *Effect of intraperitoneal injections.*—Intermittant injections of the immune serum were given intraperitoneally to a group of 8 animals. Following the injection of 0.1 c.c. of the serum, a slight fall was observed in the red cell and hæmoglobin content, 24 hours after the injection. At this stage, the average red cell diameter was found to be reduced as seen in peripheral blood smears, so that minimal anisocytosis was observable. By the 4th day, however the anæmia became well established and with the prolonged administration of the serum, a progressive decrease occurred in the red cell number and hæmoglobin of the blood. Peripheral blood smears now showed a greater degree of anisocytosis. Reticulocytes appeared in large numbers followed by nucleated red cells. As the administration of the serum was discontinued, the blood changes tended to return towards normal and following a period of rest for 8 days, the red cell and hæmoglobin had attained their pre-injection value. Likewise with an increase in the dose of the serum from 0.1 c.c. to 0.25 c.c. and finally to 0.5 c.c. the anæmia became more fulminant, the intensity of hæmoglobinuria and quantity of urinary urobilinogen excretion increased.

A significant observation in this phase was the retardation that occurred in the regenerative activity of the bone marrow following an increase in the dose of the serum. As the anæmia increased in severity, the number of reticulocytes, instead of increasing in number, showed a progressive fall. Nucleated erythrocytes, instead of increasing in number, showed a progressive fall. Nucleated erythrocytes were absent from the blood smear. The mean leucocytes count likewise, decreased from 22,000 to 8,000.

Effects of intramuscular injections.—Effects of the administration of immune intramuscularly were studied in 30 guinea-pigs. These animals developed a chronic hæmolytic syndrome, which was slow in its onset, characterized by a protracted course and a well-marked phase of regeneration of the bone-marrow. The fall in the number of red cells and hæmoglobin was observed on the 5th day of the injection, and severe anæmia was discernable 10 days later. A significant individual variation was noted in the response of the animals to an identical dose of the hæmolytic serum. By the end of 3rd week, 7 out of group of 10 animals had died. The surviving animals relapsed into a chronic anæmia state, where the destruction of the red cells seemed to have been balanced by the regeneration in the bone-marrow. Subsequent administration of hæmolysin to these animals thereafter, for periods up to 30 days, did not alter their hæmatologic status quo.

Another significant observation in the animals of this group was the appearance of small, thick red cells (microspherocytes). These cells were seen at the earliest 24 hours after the injection and were prominent in the blood smears from 3rd to 6th day. A few days after the development of microspherocytosis, intense reticulocytosis took place. The number of reticulocytes increased from an average of 2 to 32 per cent. This was followed by appearance of nucleated red cells in numbers in the peripheral blood smears. In certain instances as many as 42 nucleated red cells were counted per 100 leucocytes. The blood picture now showed pronounced anisocytosis, characterized by the presence of very large polychromatophilic cells lying side by side with microspherocytes, the latter having considerably decreased in number.

Changes in the shape and size of the red cells were made by the study of the Price Jones curves. It was observed that a fair degree in the red cell diameter (from 7.5 to 6.1 μ) occurred on the 3rd day of the administration of immune serum. This was accompanied by an increase in the mean red cell volume (m.c.v.), and also in the mean red cell thickness (m.c.t.), so that the Spherical Index was somewhat increased. Associated with the volume changes in the red cells was observed an increase in the osmotic fragility also. As the administration of the serum was prolonged the above changes progressed in magnitude, and by the 6th day, the red cells attained their highest Spherical Index of 0.60. At this period the osmotic fragility of the red cells was seen to register a greater increase. With the Spherical Index reaching a value of 0.6 (normal 0.27), red cell lysis was noted to start even in 0.8 per cent saline.

Price Jones curves drawn from this period onwards showed a very broad base and number of humps on the right side of the peak. This would indicate that a greater variation was present in the size of red cells, as was also evinced by examination of blood smears, and that there were present populations of immature cells having much greater diameter than fully mature erythrocytes.

The effect of intraperitoneal administration of hæmolytic serum was studied also in another group of 10 splenectomized guinea-pigs. It is well known that splenectomy *per se* is followed by changes both in the number and morphology of red cells. Therefore, the changes in red cell number following the removal of spleen in another group of animals, that did not receive hemolysin, were also studied. It was observed that following the injection of identical doses, the magnitude of red cell decrease is much less pronounced in splenectomized animals than in animals with intact spleen. The same was true of hæmoglobin, which ran parallel with erythrocytes. Changes in the shape and size of red cells were, likewise, more marked in non-splenectomized animals, distinctly shows that the Spherical Index of the red cells and the percentage of microcytes increased much more in animals with an intact spleen than in those subjected to splenectomy. Examination of blood smear in the splenectomized group showed, that despite a lesser degree of anæmia, the number of nucleated red cells was more as compared with animals with intact spleen. Moreover, fair number of target cells and Howell Jolly bodies were also observed in peripheral blood of splenectomized animals. Similar morphologic changes were, however, present in the group of splenectomized guinea-pig as well that did not receive hæmolytic serum.

Bone marrow.—Aspiration smears of bone marrow examined at the height of anæmia in all the groups showed marked hypercellularity. There was preponderance of cells of the erythrocytic series and the M.E. ratio was found to be considerably decreased. The cells were mainly basophilic and polychromatophilic normoblasts, many of them showing mitotic activity. At no time have we observed the bone marrow to be hypocellular. In three instances, where the animals survived and were given immune-serum for periods up to 45 days, the bone marrow still showed normoblastic hyperplasia.

Hæmagglutination.—Examination of blood for hæmagglutination showed the presence of the phenomenon in all animals that received immuneserum. The route of administration did not show any significant difference either in the magnitude or the appearance of agglutination of the red cells. The agglutination was detectible as early as 24 hours after the injection in certain cases. It was, however, invariably observed in each instance between 6 and 8 days of the administration of the serum. Thereafter, the intensity of the reaction diminished and the phenomenon was rarely observed, if at all, after 15 days.

The phenomenon was conspicuous by its absence in untreated animals. Likewise, we have failed to detect agglutination of red cells in splenectomized animals given immune serum. Only two animals formed an exception to this rule and in these, the reaction was very weak and completely disappeared after the 5th day.

Pathologic anatomy.—In most of the animals that died of hæmolytic anæmia the size of spleen was 2 to 3 times of the normal. In one instance, however, the spleen was enormously enlarged. Microscopic examination of the spleen showed extreme degree of congestion, with dilation of sinusoids. The sinusoids contained a large number of nucleated red cells besides the cells of myeloid series. There was evidence of histocytic proliferation as evinced by giant cell formation and erythrophagocytosis. Examination of the liver histologically revealed only marked congestion. We have consistently failed to observe any areas of hepatic necrosis.

Comments.—It is well known that the occurrence of many abnormal hæmatologic reactions follow an immunologic pattern. Bjorkland and Hellstrom have claimed to have produced aplasia of the bone marrow in the rabbit by use of anti-rabbit bone marrow serum. Their results still remain to be confirmed.

Our studies show that following the administration of anti-erythrocytic serum in varying amounts, the resulting anæmia is hemolytic in character and associated with hyperplastic changes in bone marrow. The fact that animals receiving immune serum developed anæmia of variable degree, while those that received normal serum did not clearly indicate that the anæmia was due to hemolysin. We have further observed that the magnitude of hemolytic syndrome resulting after administration of the immune serum depends on its antibody content. Thus, when the serum is administered by intra-cardiac route or in increased amounts, a fulminant anæmia develops. Similarly, when smaller doses of serum are employed, the resulting syndrome is of a sub-acute nature, characterized in its course by an earlier phase in which

the red cell diameter is generally reduced and later on, by the development of marked reticulocytosis. Small doses and intramuscular injections of the serum result in a chronic type of anæmia in which, following an initial phase of minimal hæmolysis, the blood picture is later on dominated by regenerative activity of the bone marrow.

The course of the development of anæmia in experimental animals, as shown by us, bears a close resemblance to that observed in human beings. It is recognized clinically also that hemolytic anæmias in man may be acute, sub-acute or chronic. The course of the anæmia observed in the group of animals injected by intracardiac route is comparable to those human cases that are observed in acute hemolytic crisis. These animals showed, besides a rapid fall in the red cell count and hæmoglobin, an increased excretion of fæcal urobilinogen associated with rapid rise in serum bilirubin level. Hæmoglobinuria was a marked feature. We did not, however, observe evidence of bone-marrow regeneration which is an accepted feature of hemolytic crisis in men. On the contrary, in our animals, there was minimal reticulocytosis and the mean leucocyte count fell from 22,000 to 8,000. Bone marrow showed hypoplasia during this period. More recently, it has been recognized that in many cases of spherocytosis in crisis, there is disappearance of reticulocytes from the blood, leucopenia and thrombocytopoenia. These changes have attributed to temporary arrest of bone-marrow activity. What relationship this 'regenerative crisis' bears to our observations in experimental animals is not clear.

The development of anæmia, as shown by us, falls in three distinct phases. In the first phase, volume changes take place resulting in the formation of thicker red cells that have decreased m.c.d. at the cost of their surface area. The second phase is characterized by the occurrence of the hemolytic process with consistant changes in the peripheral blood picture and blood chemistry. The third phase is that of regeneration. The ultimate picture of the resulting syndrome is dependant on the balance between the second and third phases.

28. Investigation into the problem of carrier state of the virus of hepatitis under Dr. B. K. Aikat at the S. S. Karnani Memorial Hospital, Calcutta.

The investigations were carried out according to the approved plan. As desired by the Sub-committee, Dr. Work of the Virus Research Centre, Poona was consulted by the workers and his suggestions have been implemented.

One of the methods suggested in the original scheme—the skin test—for identification of carriers was abandoned. Dr. Work took great pains to ascertain the usefulness of the test from workers in the States and after many personal communications, advised against its use. The possibility of using a complement—fixation test using the serum of patients in the acute viræmic stage of the disease as an antigen was suggested.

It is apparent that one has to depend on chance finding to obtain the serum in the acute viræmic stage and the difficulties of performing

the test with undiluted serum as an antigen will be evident from the results. Dr. Work has been requested to obtain greater details of the technique that is being employed in the States. Attempts are also being made by us to modify the technique which has been used but with inconclusive results.

The approach to the problem was, therefore, limited to the use of battery of liver-function tests and to try and study the liver biopsy, where possible, in those cases showing a significant but persistent abnormality in the screening tests.

It has been possible to perform liver biopsies in only 4 suspected blood donors and 2 mothers of infants suffering from infantile cirrhosis. Two of the blood donors and one of the mothers showed evidences of hepatic pathology of the nature of smouldering hepatitis and the other two donors and one mother showed portal scarring and infiltrates.

Electrophoretic pattern of serum proteins and cholinesterase estimations were introduced with great advantage.

In a suspected case of liver disease the changes in electrophoretic pattern appear to be a reliable guide of hepatic damage. It is too premature to draw any conclusions from the result so far obtained. It will, however, be pertinent to suggest that there are sufficiently suggestive pointers to extend the enquiry to larger numbers of cases before coming to any definite conclusions. In the absence of feasible methods of identification of the virus, this appears to be a fruitful indirect approach which may have great possibilities.

TABLE I

Total number of cases in different groups of cases showing abnormality in hepatic function tests.

Groups	Total number	CASES SHOWING ABNORMALITY IN HEPATIC FEVER TEST :
		Number
Normal adults	100	3
Mothers of marsmic children	15	1
Postnatal mothers	76	6
Blood donors	100	12
Antenatal mothers	73	9
Mothers of infantile cirrhosis children	15	9
Infectious hepatitis	36	36
Cirrhosis of liver	42	42
Total	457	

TABLE II

Results of abnormal hepatic function tests in the groups cases.

One test positive	Normal adults	Mothers marmmic children	Post-natal mothers	Blood donors	Ante-natal mothers	Mothers of infantile cirrhosis
Thymol turbidity	3	1	3	9	3	9
Zn sulphate turbidity	2	...	2	4	3	7
Cephalin cholinesterase	2	1	3	4	2	7
Serum bilirubin	...	...	1	3	2	3
Alkaline phosphatase	...	...	...	2	1	3
Plasma cholinesterase	...	...	...	...	1	...

II (a)

Tow tests positive	Normal adults	Mothers marmmic children	Post-natal mothers	Blood donors	Ante-natal mothers	Mothers of infantile cirrhosis
Thymol turbidity	2	...	2	4	2	7
Zn sulphate turbidity						
Thymol turbidity cephalin cholesterol flocculation	2	1	3	4	3	7
Zn sulphate turbidity serum bilirubin	...	...	1	3	1	2
Serum bilirubin alkaline phosphatase	...	...	1	2	1	2
Plasma cholesterase thymol turbidity	...	...	...	...	...	1
Plasma cholinsterase Zn sulphate turbidity	..	...	...	...	...	1

TABLE III

Results of other hepatic function tests in cases of cirrhosis showing abnormal electrophoretic pattern.

One test positive	Total number of tests done	Per cent of positive
Alkaline phosphatase	42	50
Serum bilirubin	42	54
Zn sulphate turbidity	42	61·5
Thymol turbidity test	42	64·5
Cholinesterase	42	83·3

III (a)

Two tests positive	Total number of tests done	Per cent of positive
Zn sulphate turbidity thymol turbidity test	42	72
Alkaline phosphatase serum bilirubin	42	80
Plasma cholinesterase Zn sulphate turbidity	42	88
Plasma cholinesterase thymol turbidity	42	94

TABLE IV

Results of other hepatic function tests in cases of infectious hepatitis, showing abnormal electrophoretic pattern.

One of the tests	Total number of tests done	Per cent of positive
Plasma cholinesterase	36	70
Serum bilirubin	36	82
Alkaline phosphatase	36	90
Zn sulphate turbidity	36	90
Thymol turbidity	36	9

IV (a)

Two tests positive	Total number of tests done	Per cent of positive
Plasma cholinesterase Zn sulphate turbidity	36	90
Alkaline phosphatase serum bilirubin	36	90
Plasma cholinesterase thymol turbidity	36	95
Zn sulphate turbidity thymol turbidity	36	98

TABLE V

Complement fixation test in virus hepatitis

Serial number	Serum of pt. (1 in 5)	Ant. undil.	Dose comp.	Ant-control	Serum control	Result of C. fix. test	Remarks
1	a	i	3M.H.D.	—	—	+	CF test positive
2	b	ii	2M.H.D.	±	—	±	Doubtful
		iii	3 M.H.D	±		±	"
		iv	"	±	—	±	"
		v	"	±	—	±	"
3	c	vi	"	±	±	±	"
		vii	"	±	—	±	"
4	d	viii	"	±	—	±	"
		ix	"	±	—	±	"
		x	"	±	—	±	"
5	e	xi	"	±	—	±	"
		xii	"	±	—	±	"
		xiii	"	±	—	±	"
6	f	xiv	3M.H.D.	±	—	±	"
		xv	"	±	—	±	"
7	g	xvi	"	±	—	±	"
		xvii	"	±	—	±	"
8	h	xviii	"	±	—	±	"
		xix	"	±	—	±	"

29. Investigations to find out the biochemical changes and effect of different types of therapy in hepatic coma under Dr. N.R. Konar at the N.R.S. Medical College, Calcutta.

Thirty cases of hepatic coma have so far been observed. The primary diseases of the liver giving rise to hepatic coma were: Portal cirrhosis of liver 11, infective hepatitis 9, infective hepatitis superimposed on cirrhosis of liver 4, carcinoma liver 3, biliary cirrhosis 1, splenomegalic cirrhosis 1, and post-operative jaundice 1. In 24 cases the onset of coma was insidious, in 5 the coma was precipitated by hæmatemesis and melæna and in one by abdominal paracentesis. Drowsiness was present in all the thirty cases. In addition, restlessness was present in 12, hiccough in 3, convulsion in 1, and cholæmic cry in 1. Hepatic factor was not observed in any of the cases. Flapping tremor was present in two cases. There were no uniform neurological signs in these cases. Extensor plantar response was present in 14, exaggerated deep reflexes in 18 and ankle clonus in 13 of the cases. Urea and NPN content of blood was high in 22 and normal in 8 cases; cholesterol content of serum was low in 24, normal in 5 and high in 1 and chloride content of blood was low in 17 and normal in 13 and bilirubin content of serum was high in 28 and normal in 2 cases. In 3 cases ammonia level of blood was determined and found to be raised. There was lowering of blood ammonia level in these cases after intravenous administration of sodium glutamate, and along with it the mental condition of the patients improved. Though the coma was reversed in 5 out of 30 cases only one, patient survived.

30. Inquiry on blood and CSF ammonia and glutathione (GSH) in liver disease, with or without coma and the effect of glutamate on these levels and the clinical condition under Dr. Shiv Kumar and Dr. K.L. Wig at the Medical College, Amritsar.

Report on the work done on the enquiry: blood and C. S. F. ammonia and glutathione (GSH) in liver diseases with or without coma and the effect of glutamate on these levels and clinical condition, up to 31st December, 1956.

1. Blood glutathione (GSH) estimations were done on 36 healthy adults, history of liver diseases being excluded. In each case blood for estimation was collected from the antecubital vein during the post absorptive-phase, after half-an-hour rest in the Department at 9 a.m.

Glutathione (GSH) was determined by the method of Woodward and Fry (1932). 0.01 g. of very fine powdered potassium oxalate, instead of a solution, was added to 3 c.c. of blood. Estimations were performed in duplicate.

The analysis of results is given below :—

<i>Blood glutathione (GSH)</i>			
Number of observations	...	36	
Range	...	22.00 to 51.28	mg. per 100 c.c. blood
Mean	...	32.69	mg. per 100 c.c. blood
Standard error of mean	...	± 1.03	
Standard deviation	...	± 6.14	
Coefficeint of Variation	...	18.8	per cent

2. The cerebrospinal fluid (C.S.F.) glutathione (GSH) estimations were carried out on 18 healthy adults who were admitted to the Surgical Wards of the V. J. Hospital, Amritsar, for elective surgery. The cerebrospinal fluid specimen was obtained in the operation theatre before the induction of spinal anæsthesia. Five to ten c.c. was collected. The estimations were performed in duplicate by the method of Woodward and Fry (1932). No anticoagulant was added. The analysis of results is given below :—

<i>C.S.F. glutathione (GSH)</i>			
Number of observations	...	18	
Range	...	1.227 to 8.589	mg. per 100 c.c. C.S.F.
Mean	...	3.927	mg. per 100 c.c. C.S.F.
Standard error of mean	...		
Standard deviation	...	± 1.816	

3. Work on ammonia estimations could not be started due to non-receipt of equipment, viz., Conway Units and Burettes from the United Kingdom despite our best efforts. However, 36 Conway Units were received on 8-10-56. In the end of November, 1956 burettes were received and the standardization of apparatus was started. This work has been completed and in January 1957, work will be started on clinical material.

31. Electrophoretic and chromatographic studies in experimental animals and human beings in health and liver injury under Dr. M. Balasubrahmanyam and Dr. Ramji Dass at the Medical College, Patiala.

The object of the work was to study the serum proteins by electrophoresis and correlate the findings with chromatographic analysis of amino acids in serum and liver tissue in human cases of liver injury and in experimental liver injury.

Eighteen cases of infective hepatitis and 17 cases of cirrhosis of the liver have been studied. Experimental liver injury is produced with carbon tetrachloride, beryllium and high-fat diet in different groups of albino rats.

Serum electrophoresis is carried out by the horizontal paper-electrophoresis technique using borate barbitone buffer. The stained paper is scanned through a photo-electric densitometer.

Pieces of liver are extracted repeatedly with 70 per cent alcohol and the extract dried. The dried extract is re-dissolved in butanol acetic acid, water mixture and subjected to double dimensional paper chromatography. The amino-acid spots, after development, are eluted and the quantity estimated using pure amino acids as standards.

An attempt is made to correlate the findings in the above procedure.

- 32. Study of environmental and nutritional factors affecting the incidence of atherosclerosis and coronary heart disease in Indians under Dr. K. S. Mathur and Professor P. N. Wahi at the S.N. Medical College, Agra.**

CLINICAL MATERIAL

Three groups of cases are being studied :

Group 1.—Cases of acute myocardial infarction as they are definitely known to suffer from coronary atherosclerosis. All cases admitted to S.N. Hospital and some private cases are investigated and records maintained on *pro forma* 1.

Group 2.—Cases of hypertension and diabetes, which are known to predispose to atherosclerosis and coronary artery disease. These are hospital cases, and also those who are picked up from group 3. The same *pro forma* is used for these cases as for group 1.

Group 3.—Persons between 25 and 55 years of age were collected at random. Unselected groups of people from different social and economic status and from different occupations were examined. Teachers from Agra College and St. John's College had been examined in their respective colleges; the lists of teachers of other colleges had been obtained and will be taken up after Dasehra vacations. The examination of worker in Indra Spinning Mill has been started in the mill area. The list of all factory workers in the age group 25 to 55 years from amongst the 8,000 workers registered with the two Government dispensaries attached to Health Insurance Scheme, has been prepared. The third category of persons examined in this group is clerical workers. All these cases are being studied on *pro forma* 2.

METHOD OF INVESTIGATION

The cases belonging to groups 1 and 2 are either admitted to the hospital or are brought there for detailed history, and examination

including electrocardiogram, screening and pathological and biochemical tests. Group 3 people are examined at their respective places of vocation and samples of blood are collected and taken to laboratory in the hospital for examination. In case of suspicious findings or family history of coronary or cerebral atherosclerosis or hypertension they are asked to come to the hospital for further check-up.

All the three groups of people are interrogated in detail about their diets. Fat and caloric value of the diet are calculated in accordance with the tables given in Heart Bulletin No. 23 'The Nutritive Value of Indian foods and the Planning of satisfactory diets. by Patwardhan and Ranganathan (1956).'

The cholesterol content of the diets is calculated from Diet Manual.

An attempt is made to determine separately the amounts of animal and vegetable fats ingested, as the former has high cholesterol content.

The serum cholesterol is estimated by the method Albert Bennie and Albert (*Jour. Lab. & Clin. Med.* **41**, 486, 1953.) The phospholipids are estimated by the method of Young Burg (Practical Physiological Chemistry by Hawk and Summerson).

OBSERVATIONS AND RESULTS

(A) The ten-year (1946-1955) analysis of cases of myocardial infarction treated by one of us (K.S.M.) has not been completed yet. The first 110 cases seen during the period 1946-1949 have been analysed. The ages of patients range from 25 years to 75 years—25 to 35 years (9 cases), 36 to 45 years (22 cases) 46 to 55 years (41 cases), 56 to 65 years (30 cases), and 66 to 75 years (8 cases). This included 14 females, and rest were males. Thirty patients were heavy weight, 72 average built and 8 were thin and underweight. Social status was classified as upper class 40 cases, middle class 54 cases and lower income group 16 cases. Only 5 came from villages, the rest were town-dwellers. Seventy-two of the patients were vegetarians and 38 non-vegetarians. Only 22 were smokers and 16 took moderate quantities of alcohol. Twenty-two were businessmen, 11 doctors, 5 zamindars, 7 clerks, 2 teachers, 4 police officers, 3 engineers, 2 lawyers, 6 shopkeepers, 2 farmers, 11 government servants in different services, 3 administrators. Co-existent hypertension was present in 46 cases and diabetes in 19 cases. Acute attacks were precipitated most in winter months—January (13 cases), February (18 cases), March (8 cases), April (7 cases), May (7 cases), June (3 cases), July (10 cases), August (10 cases), September (9 cases), October (11 cases) and December (7 cases). Analysis of the remaining cases will be completed during the coming months.

(B) Since the beginning of this year, 14 cases of acute myocardial infarction (group 1) and 16 cases of hypertension and diabetes (group 2, have been studied. The number is too short for any detailed analysis both with regards to dietary fats, cholesterol and calories and blood lipids.

Eighty-seven teachers, 13 clerks and 10 mill workers have so far been examined in group 3. University teachers from Agra College and St. John's College were all males ranging in age from 25 to 55 years. Five of them had raised blood pressure and they are being investigated

in detail. None of them had clinical evidence of atherosclerosis. The history of one of them is worth recording. He is a Hindu male, aged 47 years. He has seven brothers, six of them are hypertensive and diabetic, two of them died suddenly at the age of 55 years and two others were diagnosed as cases of acute myocardial infarction. His own blood pressure is 142/104 with normal size heart and normal electrocardiogram. He is being investigated in more detail and will be followed from year to year. The diet of the university teachers is rich in fats (minimum 71.6 g., maximum 358.9 g. with an average of 82.4 g. daily), cholesterol (minimum 80 mg. maximum 386.9 mg. with an average of 279.1 mg. daily) and calories (minimum 1280.5, maximum 3528.5 with an average of calories per day). Serum cholesterol and phospholipids were done in 24 cases. The average levels of serum cholesterol were 291.9 mg. per 100 c.c. serum (minimum 197 mg. maximum 420 mg.) and phospholipids 261.00 mg. per cent (minimum 242.5 mg. maximum 282.5).

The ten labourers examined in Indra Spinning Mill were all underweight and undernourished with an income of Rs. 30 to Rs. 50 per month. Their ages ranged between 25 and 50 years. Their diet is very poor in fats (minimum 15.4 g. maximum 23.8 g. with an average of 20.5 g. daily), cholesterol (minimum 4 mg. maximum 153 mg. with an average of 41.7 mg. daily and calories (minimum 865.3, maximum 1950 with an average of 1,766 calories daily). Serum cholesterol and phospholipids of all the ten cases were done. The average serum cholesterol level was 199.5 mg. per 100 c.c. serum (minimum 110 mg., maximum 250 mg.) and phospholipids 232.5 mg. per cent (minimum 207.5 mg. per cent, maximum 250.0 mg. per cent).

Thirteen clerks from Agra College office have been examined so far. None of them showed any evidence of hypertension or atherosclerosis. The analysis of their diets gives the daily quantities of fat (minimum 40.7 g. maximum 117.9 g. with an average of 62.1 g. cholesterol (minimum 69.8 mg., maximum 341.4 mg., with an average of 190.1 mg. and calories (minimum 1,223, maximum 2,421.6, average 1,971 calories). The blood was examined in 6 cases with an average serum cholesterol level 254 mg. per 100 c.c. serum (minimum 175 mg. maximum 360 mg.) and phospholipids 243.5 mg. per cent (minimum 235.5, maximum 252.5).

The work on this inquiry has just started. The factual data so far collected have been given but no conclusions can be drawn.

TABLE I
Diet analysis group 3 cases.

	Number examined	Fat	Cholesterol	Calories
University teachers	87	82.3 g. (71.6—358.9)	279 mg. (80—386.9)	2,232 calories 12,805— 3,528.5
Labourers	10	20.5 g. (15.4—23.8)	41.7 mg. (4—153)	1,766 (865.3 —1,950)
College clerks	13	62.1 g. (40.7—117.9)	190.1 (69.8—341.4)	1,971 (1,223 —2,421.6)

TABLE II

Blood lipid studies in group 3 cases.

	Number examined	Serum cholesterol	Phospholipids (lecithin)
University teachers	24	291.9 mg. (197—420)	261.0 mg. (242.5—282.5)
Labourers	10	199.5 mg. (110—250)	232.5 mg. (207.5—250.0)
College clerks	6	254 mg. (175—360)	243.5 mg. (235.5—252.5)

33. Study of the pattern of coronary circulation by injection technique with special reference to coronary heart disease under Dr. P. N. Wahi and Dr. K.S. Mathur at the S. N. Medical College, Agra.

The aim of the inquiry is to study the pattern of coronary circulation in the different economic and social groups of North Indians and analyse its relationship with the incidence of coronary heart disease in these groups. The study is, therefore, designed to (a) demonstrate the normal and abnormal patterns of coronary circulation, and (b) to study the correlation of the incidence and distribution of myocardial damage with the extent and nature of anastomosis and coronary narrowing and occlusion.

Technique.—The injection technique of Schlesinger (*Amer. Heart Jour.*, **15**, 528, 1938), with certain modifications, was used as it has been found to be simple practical, quick and inexpensive. This technique has also the added advantage that there is no overlapping of the arteries on the roentgenogram, unlike those attending the interpretations of three dimensional specimen from a two dimensional skiagram of earlier workers. By this technique the heart muscle also is available at the end for the study of its correlation with coronary artery changes.

Apparatus.—The apparatus consists of two parts (1) a warm saline bath, of 6 gallons capacity, the temperature being maintained at 44°C. by means of a thermoregulator and air-blower, (2) a mercury manometer consisting of two manometers connected by a three way stopper and to the tubes of the injection mass in the saline bath.

Injection.—The right and left coronary arteries are dissected as they leave the aorta, a glass cannula is passed in each of them and tied securely. The heart is immersed in the warm saline bath and the coronary arteries are washed with normal saline, injected at 150 mm. Hg. pressure. Then the arteries are injected simultaneously with the warm radio-opaque lead phosphate agar mass at 150 mm. Hg. pressure. The agar mass through each artery is differently coloured (by addition of dyes). The direction is reversed after 5 to 7 minutes so that the mass enters the large size anastomatic vessels. The heart is then transferred to a iced-normal saline bath for 30 minutes, so as to solidify the injection mass.

Dissection.—The heart is then unrolled by a series of six incisions as advocated by Schlesinger (*loc. cit.*). Following this the heart is x-rayed placing the pericardial surface in contact with the x-ray film and employing the following technique.

Tube XP-1 fine focus. distance 42 inches, milliamperes 20 KV 48 medium conc. till one second plain folder. Guided by the roentgenogram, arteries are dissected. A drawing made from x-ray appearance is marked with narrowing, calcification, occlusions and anastomosis as they are met with during dissection. Blocks are made from different parts of the myocardium and sections studied under microscope to correlate them with arterial changes.

The amount of work done so far employing this technique is not very appreciable as the research worker joined only on 29th June, 1956 and as time was also taken in designing and making the apparatus. The work has started in right earnest and six hearts have been injected so far.

34. Inquiry on the diagnosis of pre-symptomatic diabetes and hypertension under Dr. K.S. Sanjivi at the Madras Medical College, Madras.

Objects of the work.—The study was undertaken to see if there is any difference in the response of healthy young men coming from families. (a) with a history of diabetes, (b) with a history of high blood pressure, and (c) with no such family history, in their clinical and biochemical reactions to a standard dose of 'cortisone' administered over a 7-day period.

As the work was started late it is too early to say anything definite about the results of the study.

35. Inquiry on anæmias in infants and children under Professor H.I. Jhala, Dr. B.D. Patel, Dr. J.G. Parekh, Dr. C.G. Soraiya and Mr. N.C. Dutt, at the Grant Medical College, Bombay.

With a view to collect data on hematological standards of infants born to healthy mothers, clinically healthy mothers not exceeding 4th parity were selected from the antenatal clinic of the J.J. Group of Hospitals. Investigations included clinical examination, screening of the chest to exclude tuberculosis, examination of peripheral blood to estimate hemoglobin, erythrocyte and leucocyte counts, as well as determination of various indices, Rh grouping, biochemical examination of blood to ascertain levels of plasma proteins, serum iron and vitamin B₁₂, serological examination for syphilis by VDRL test and RMT, and routine examination of urine. All the selected patients' dietary intake was evaluated by close questioning by the health visitor. Enquiries were also made into the living and economic conditions of the family.

These cases were followed up monthly/fortnightly at the antenatal clinic and attention was paid to exclude physical and urinary abnormalities. At full term the mothers were requested to come to the hospital for delivery so that their infants' blood could be collected for investigations.

So far, 73 antenatal cases have been investigated, and all of them were clinically healthy. There were 25 primipara, 19 cases of para II, 11 of para III and 18 cases of para IV. Sixty-nine per cent of the cases had hemoglobin levels above 10 g. per cent. The whole group of antenatal cases were classified arbitrarily into four groups according to the hemoglobin levels as follows :

Group I with hemoglobin 12 g. per cent and over had 17 cases with mean hemoglobin 12.2 g. per cent r.b.c. 4.1 mill./cmm., p.c.v. 36.7 per cent and m.c.h.c. 33 per cent.

Group II with hemoglobin 11 to 11.9 g. per cent had 14 cases with mean hemoglobin 11.3 per cent r.b.c. 3.7 mill./cmm., p.c.v. 35.2 per cent and m.c.h.c. 32 per cent.

Group III with hemoglobin 10 to 10.9 g. per cent. had 15 cases with mean hemoglobin 10.41 per cent. r.b.c. 3.68 mill./cmm., p.c.v. 33.9 per cent and m.c.h.c. 31 per cent.

Group IV with hemoglobin under 10 g. per cent. had 27 cases with mean hemoglobin 8.7 per cent r.b.c. 3.67 mill./cmm., p.v.c. 31.12 per cent and m.c.h.c. 28 per cent.

Results of classification on a morphological basis were as follows :

Normocytic normochromic 43 cases. normocytic hypochromic 13 cases. microcytic normochromic 7 cases, macrocytic hypochromic 5 cases. microcytic normochromic 2 cases, and microcytic hypochromic 3 cases.

Of the 73 cases studied, 38 have delivered in this hospital and cord blood could be collected from 22 infants. In 10 of these blood was examined for their hematological values. The others were unsuitable for hematological study. Serum iron and serum vitamin B₁₂ was estimated in all the 22 cases. The infants were classified into four groups corresponding to their mothers groups (by hemoglobin levels as mentioned above). Cord blood findings were as follows :

Group I had mean r.b.c. 5.84 mill./cmm., hemoglobin 18.25 per cent, p.c.v. 58.75 per cent, m.c.h.c. 31 per cent, and m.c.v. 101u³.

Group II had mean r.b.c. 5.34 mill/cmm., hemoglobin 17.3 g. per cent. p.c.v. 54 per cent, m.c.h.c. 32 per cent, and m.c.v. 101u³.

Group III had mean r.b.c. 5.6 mill./cmm., hemoglobin 16.5 g. per cent. p.c.v. 54 per cent, m.c.h.c. 29 per cent and m.c.v. 104u³.

Group IV had mean r.b.c. 5.28 mill./cmm., hemoglobin 16.1 g. per cent, p.c.v. 52 per cent, m.c.h.c. 31 per cent and m.c.v. 98u³.

All the infants heel blood was examined routinely on the sixth day of life. Mean values of findings from the heel blood were :

Group I: R.b.c. 5.97 mill./cmm., hemoglobin 19.3 g. per cent, w.b.c. 10,500/cmm.

Group II: R.b.c. 5.78 mill./cmm., hemoglobin 17.6 g. per cent, w.b.c. 10,700/cmm.

Group III: R.b.c. 5.84 mill./cmm., hemoglobin 16.3 g. per cent, w.b.c. 9,600/cmm.

Group IV: R.b.c. 5.7 mill./cmm., hemoglobin 15 g. per cent, w.b.c. 9,600/cmm.

II. Selection of infants and children was done from the Children's Out-patient Clinic as follows:

(a) Those who had obvious pallor were first checked for their hemoglobin. Those having less than 7 g. per cent were admitted to the ward for the detailed study, including clinical examination, examination of blood for hematological values, serum iron and vitamin B₁₂ levels, fetal hemoglobin estimation, Rh grouping and examination of bone marrow aspiration, examination of stools and urine, screening of the chest and Mantoux's test. The dietary history was recorded by the health visitor.

(b) Patients who had hemoglobin more than 7 g. per cent. were investigated and treated as outpatients.

Fifty-five children have been investigated. Twenty-five were treated as in-patients and the remaining 30 were treated as out-patients.

(a) *Results of investigations of in-patients.*—Presenting symptom was fever in 14 cases, swelling of legs in 13, diarrhoea in 11, weakness in 10, loss of appetite in 8, breathless in 3 and cough in 3 cases.

Important physical signs were enlargement of liver in 7, splenomegaly in 4, edema in 4, cardiac enlargement in 4, cardiac murmur in 6 and dehydration in 5 cases.

Associated and etiological conditions were: Malnutrition in 11 cases, gastro-intestinal infection in 9, lung infection in 7, round-worm infection in 6, hookworm in 1, tuberculosis in 2, Kala Azar in 1 case and anæmia alone was found in one case.

Anæmias classified on a morphological basis showed: Macrocytic normochromic in 9 cases, macrocytic hypochromic in 9, normocytic normochromic in 15, normocytic hypochromic in 7, microcytic normochromic in 5 and microcytic hypochromic in 10 cases. Bone-marrow study in 40 cases revealed that 15 presented a dimorphic picture, 22 showed normoblastic reaction, while in 3 cases there was megaloblastic hyperplasia. One marrow showed L.D. bodies.

Treatment was guided by the results obtained from the investigations. Patients with infection or infestation of worms were treated with appropriate therapeutic agents along with hematinics. Iron was administered intravenously to 8 children, the total dose being calculated and given in divided doses. No untoward reaction was observed. Blood transfusion was given at the commencement of therapy 5 patients where the degree of anæmia was severe.

Folic acid was given parenterally to cases of megaloblastic anæmia. Children of the younger age group were given iron orally in the form of ferrous gluconate. Children with anæmia and malnutrition were treated with a high-protein diet consisting of proteins 74 g., fat 71 g., carbohydrate 213 g., and a total caloric value of 1,716.

The outpatient series have been studied with the main object of ascertaining the incidence of the various types of anæmia. It has been observed that regular therapy and follow-up is not possible amongst this group of patients.

36. Experimental study of pancreatitis under Dr. P.N. Chuttani at the Government Medical College, Patiala.

The hepato-pancreatic system in monkeys was studied. After preliminary normal serum amylase and urinary diastase value studies, the pancreatic duct was ligated under nembutal anæsthesia. The animal died after 8 hours. Histo-pathological studies are in progress.

Due to difficult position of supply of monkeys, work was started on dogs. Three dogs have been operated upon so far, after preliminary baseline studies, and pancreatic duct ligated. Histopathological studies are in progress.

37. Development of a simple method of preserving or preparing aortic and arterial homografts under Dr. Timothy Takaro at the Wanless Tuberculosis Sanatorium, Wanlesswadi.

1. *Insertion of grafts into abdominal aorta.*—Fifteen dogs were used in the formalin phase of the work. Seven animals survived three months or more. The condition of the graft in all but one was poor, and in one dog, it was fair. The graft was either thick and partially calcified, with rough pink splitting intima, or ruptured (after three months) at the suture line, or aneurysmal (heterologous formalin-fixed monkey graft). The single fair specimen seen after 13 months implantation, was fairly normal-looking, with normal feel, elasticity, and appearance, except for a 2 cm. square area of rough, red, cracked intima. One formalin-fixed nylon graft was in fair condition, and patent, after 9 months, with slight calcification.

The braided nylon graft work has been started only during the past two months (from July 1, 1956). So far, five animals have had grafts inserted with three longer survivals. Two are still alive. From early appearances, and from the one long-term formalin-fixed nylon-polythene graft, it is felt that cloth tubes of nylon, which are available in all sizes, and readily stored, meet the needs of economy and simplicity in India better than formalin-fixed homografts.

2. Three attempts to insert grafts in the thoracic aorta, without the use of hibernation, failed.

It is proposed to continue the work of implanting segment of braided nylon tubes into the abdominal aorta for long term survival

studies, and to extend the work to include the thoracic aorta, by means of a special vessel couple of our own design, which will permit rapid coupling of vessel and graft, with only very brief interruption of blood flow, and allow blood to flow through the graft, while it is being sutured.

38. Inquiry on designing of instruments and methods for their use for obtaining tissues after death without autopsy using viscerotome-like techniques under Professor H.I. Jhala at the Grant Medical College, Bombay.

With basic viscerotome, it is possible from a single midline incision of 1" to get a blind approach to the liver and spleen. Blind technique did not succeed with the other organs, using the same incision. Improvements on the instrument are under way and details will follow in regard to those that prove successful in subsequent report to them. Ninety fresh bodies were available for study, so far, for trial.

39. Biomicroscopical study of the conjunctival vessels in relation to the general arteriosclerosis and coronary artery disease under Dr. K.N. Mathur, Dr. K.S. Mathur and Dr. P.N. Wahi at the S.N. Medical College, Agra.

Object of research.

1. To establish a pattern of conjunctival vessels in cases of generalized arteriosclerosis and coronary heart disease and compare it with normals of the same age group.

2. To study the elasticity of the conjunctival vessels by measuring the calibre of the vessels and its extent of dilatation under effect of Prisol in cases of arteriosclerosis and coronary heart disease and compare it with normals.

3. To find out the elasticity of the conjunctival vessels and correlate it with the retinal blood vessels, general arteriosclerosis, coronary artery disease, blood cholesterol levels, and hypertensive findings.

4. To evaluate, if possible, a criteria to indicate an early diagnosis of general arteriosclerosis and coronary heart disease.

Conclusion.

Forty normal cases and four cases of coronary heart disease have been examined.

1. With the advance in age the tortuosity and loopings of the conjunctival vessels increase.

2. Sharp bends increase with the rise in blood pressure.

3. The calibre of the conjunctival vessels decreases with the increase in age.

4. With the advance of age the extent of dilatation of the conjunctival vessels decreases and the vessels take longer time to reach the maximum dilatation and the return to normal is also delayed.

40. Histo-pathological study of trachoma in relation with the stage of the disease under Dr. Pashpati N. Wahi and Dr. P. N. Awasthi at the S. N. Medical College, Agra.

CLINICAL MATERIAL

The clinical material is provided by the In-patient and Out-patient Departments of the S. N. Hospital, Agra. The medical students have also volunteered to act as control cases. 6 groups of cases are being studied, based on the 4 clinical stages of Maccallan.

(1) Asymptomatic cases.—(Preclinical stage)

Patient did not complain of any symptoms. In some cases there were definitely clinical findings *i.e.* dullness of the upper fornix with papillary hypertrophy and pannus. In other cases there was only pannus and nothing else. The side was positive for the inclusion bodies in these cases.

(2) Symptomatic cases.

Stage I —Cases showed pannus and papillary hypertrophy in the fornix.

Stage II—There were definite granules with pannus.

Stage III—Presence of Pannus granules and fibrosis.

Stage IV—This is the stage where fibrosis and sequelae are seen.

Mixed Stages—Some of the cases were not clear cut but various stages were combined in the same eye.

There is not much difficulty in diagnosing advanced cases, when secondary changes like fibrosis, trichiasis and entropion have already taken place. The difficulty arises in very early cases when no symptoms complained by patients are diagnostic of the trachoma.

In most of these cases were present follicles in the superior fornix and dullness in both the fornices with the various stage of pannus. In some cases early fibrosis was seen in the subtarsal sulcus. These signs were enough to diagnose a case clinically. So far not a single case has been without corneal pannus.

Laboratory Investigations.—Conjunctival smears—Eye is washed with norm alsaline after being anaesthetized with the anithaine (1%) drops. Superior fornix is swabed with 2% sodium carbonate solution to wipe out any discharge of mucous. 3 slides are then made. One slide from the medical part of the fornix, second from the central part of the fornix, and the third is from left part of the fornix. The secret of making the slide is that the epithelium of fornix is quietly stroked with the aid of the coverslip without producing any bleeding. The material thus collected is evenly over the glass slide. The smear is dried in the air and fixed in the absolute alcohol for 5 minutes. The fixed preparation is dipped into dilute Giemsa stain which is never older than a week. The slide is again washed with distilled water. A few drops of pure acetone are put on the smear for 5 to 10 seconds to wipe away the pigment granules.

Slide is again washed with distilled water and dried and studied under oil emersion lens. Cytoplasm of the epithelium stains very light blue and the nuclei reddish violet. The slide is studied for the inclusion bodies.

Biopsies.—The biopsies are taken both from upper and lower fornices after infiltrating the lids with 2 per cent Novo-caine. A stitch is passed just above the lid margin and the lid is everted. A chalazion forcep is applied on the everted lid so as to include the tarsal plate. A vertical incision is given on the tarsal plate and an incised piece of the tarsal plate is lifted, and with the help of the scissor a piece of tarsal plate including conjunctival epithelium is removed and fixed in 10% formal saline. The biopsy piece is processed and cut and stained with hæmotoxylin and eosine. Similar biopsies will also be taken from the conjunctivæ of the still born infants, and also of the normal cases attending surgical Out-patient for minor injuries and also of the cadavers, who are sent for postmortem.

Observations and Results.—53 cases have been registered upto 30th September, 1956. They comprised 47 symptomatic and 6 asymptomatic cases in both sexes. The age of the patients ranged from 10 to 70 years. The clinical diagnosis gave not much difficulty in well established cases. Asymptomatic and early cases presented difficulty which were finally labelled by clinico-pathological data. The following clinical types cases were encountered in 53 cases registered so far.

Clinical Types of Trachoma :

TABLE No. I

Types	Number
Asymptomatic cases	6
Symptomatic cases	47

Clinical Stages of Trachoma in 53 cases :

TABLE No. II

Types	Number
Asymptomatic cases	6
Symptomatic cases	47
Stage I	4
Stage II	15
Stage III	1
Stage IV	5
Mixed Stages	22
(a) 1 & 2	2
(b) 2 & 3	10
(c) 3 & 4	10

Histological diagnosis in 53 cases.—Histological findings in various clinical stages have been given without any conclusions, owing to less number of cases.

Asymptomatic stage.—The cases are very few in this stage so far. Changes have been noted in the epithelium and tarsal plate. The epithelium becomes squamous and many layered. Lymphocytic infiltration is generalised. Tarsal plate also showed lymphocytic infiltration and fibrosis.

Symptomatic cases :

Stage I—The changes noted in four cases are similar to the asymptomatic stage, except slight sub-epithelial fibrosis and owing to less number of cases in both, the demarcating features between the two will have to await further studies.

Stage II—In this stage the epithelium is many layered. Sub-epithelial tissue showed marked and diffused lymphocytic infiltration and the presence of Goblet cells. The vascularisation is also marked in the zone of lymphocytic infiltration. There is a slight sub-epithelial fibrosis seen. In the tarsal plate there is fibrosis and lymphocytic infiltration present.

Stage III—There was a single case in this group which showed papillary proliferation of the epithelium growing outwards.

Stage IV—In this stage epithelium showed papillary proliferation both upwards and downwards. The tarsal plate showed a scarring and epidermidisation of the glands.

Stage I & II : No biopsy could be taken due to reasons beyond our control in the two cases.

Stage II & III In this stage following histological changes have been noted.

- (1) Epithelium shows papillary proliferation both upwards and downwards.
- (2) Diffuse infiltration of the sub-epithelial tissue by lymphocytes both generalised and in clumps. The tarsal plate also showed scattered infiltration by lymphocytes.
- (3) Intra-epithelial infiltration of lymphocytes, few plasma cells and occasional polymorphs is also seen in few cases.
- (4) Epithelial cysts are also seen.
- (5) Vascularisation is marked where lymphocytic infiltration is heavy.
- (6) Scarring of tarsal plate is also seen.

Stage III & IV In this stage only late changes were seen.

- (1) Proliferation of epithelium both inwards and outwards.
- (2) Scarring of sub-epithelial tissue.
- (3) Fibrosis of the tarsal plate.
- (4) Epidermidisation of meibomian glands.

Smear examinations findings.—As described by Linder we came across two types of inclusion bodies, (1) initial bodies and (2) elementary bodies. Inclusion bodies were seen in 68 per cent. of cases. In majority of cases they were initial bodies present in the cytoplasm. The various forms of the initial bodies seen so far were large deeper blue granules, some discrete, some coalesced. away from the nucleus or surrounding the nucleus usually at one pole but sometimes on all sides. We came across elementary bodies in few cases. They were clear, distinct and demarcated from the nucleus over which they formed a cape at one pole. They were light pink in colour and paler than the nucleus. The capes were larger out of all proportions in size to the blue intra cytoplasmic initial bodies.

Clinical diagnosis of the cases.—As the control cases were being collected in Ophthalmic O.P.D. at least 6 cases who appeared to be normal and did not complain of any symptoms showed on examination of the conjunctival smears presence of inclusion bodies. The detail examination of the lids which showed dilatations of blood vessels of the fornices, and pannus was very slight and, just had started towards the cornea. The detailed history of the patients revealed that they get occasional itching of the lids only. Few cases who showed congestion of the lower fornix but no change in the upper fornix, did not turn out to be so in our cases. The reason which we think is that the lower fornix is exposed to so many external stimuli in India, which is comparatively a more dusty and sunny country. This produces mild congestion in the conjunctiva. Another very interesting feature that came up during our study is that even cases which are far advance into stages 3 and 4 associated with triachiasis and entropion also showed inclusion bodies. It leads us to think that sub-clinical stage of the trachoma continues inspite of tissues being replaced by the fibrous tissue.

Other important data observed in our biopsies is in stages of 1 and 2. There is sub-epithelial fibrosis which indicates that from the very start of the disease, it produces fibrosis. The fibrosis is thought to be present in late 3rd and 4th stages while our cases have shown even in the first stage. It appears that infection starts in deeper tissues earlier than the superficial tissues. This finding will play great part in treating cases. If we treat these cases by injecting a drug under the epithelium we may be able to eradicate the disease much earlier. The other striking features which we came across is ; that in four cases where there was early stage of pannus the lids showed no change except mild congestion in superior fornix. The history was taken and it was found that there is no other cause of irritation to produce congestion. In these cases conjunctival smears were repeatedly examined and after a month the smear became positive and superior fornix showed granules. It appears that trachomatous infection does not work uniformly, and probably in these cases infection caused pannus and later on produced changes in the fornix. The infection may be so mild that it produces pannus first and changes in fornix later on, or the infection may be focal first and later it gets diffused to all over. The cases studied so far are few and the study requires large number of cases to give authentic clinico-pathological data.

41. Inquiry on tropical pulmonary eosinophilia under Dr. M.V. Chari at the Kasturba Medical College, Mangalore, South Kanara.

It was noticed that tropical pulmonary eosinophilia is a common disorder in the district of South Kanara. It was also observed that the normal eosinophil count of the people in this part appeared to be higher than what it is in the rest of India. A preliminary work was, therefore, considered necessary to find out the average percentage of eosinophils in healthy individuals. Two medical exhibitions in which health check-ups were conducted helped in the collection of blood films from healthy adults for the purpose of differential count. During these health check-ups records were also kept of the height, weight, blood pressure reading, chest measurement, hæmoglobin percentage and pulse rate of the persons examined. A record of 1,593 healthy individuals (1,270 males and 323 females) was, therefore, available for the latter purpose. One hundred and ten blood films have been examined so far, while an equal number is still left to be examined. An analysis of this blood examination is given below.

The persons whose blood has been examined have been classified according to different age groups, e.g. 0 to 5 ; 6 to 10 ; 11 to 15, etc. The white blood cells have also been similarly grouped as 0 to 5 ; 6 to 10, etc. Studying the total eosinophil percentage of 100 males, it was observed that 28 persons only fall in the standard group of 0 to 5 eosinophil percentage, whilst the remaining are above 5 per cent. Out of this 28 more come within the 6 to 10 group. Only 56 men, therefore, show eosinophil percentage of below 10, whilst 44 persons have eosinophil count of above 10 per cent. It was also observed that 16 persons out of the 100 have eosinophil count of above 20 per cent.

The maximum number of persons whose blood films were analysed falls within the age group 21 to 40 years. In this age group (a total of 70 males), 39 persons had a count of 10 per cent and below ; 31 above 10 per cent; 10 above 20 per cent and 2 above 35 per cent. These persons whose eosinophils were found to be raised did not have any symptoms suggestive of tropical pulmonary eosinophilia.

Monocytes and basophils showed no abnormal variation. Lymphocyte and neutrophil counts were also analysed and found to have minor variations. They are not discussed here as they do not fall under the purview of this inquiry.

Since only 10 blood films of healthy females could be examined no definite conclusions can be drawn. Even here the eosinophil count was seen to be raised above 10 per cent in 6 out of 10 cases.

Analysis of 17 cases (12 males and 5 females) of tropical pulmonary eosinophilia followed at the Government Headquarters Hospital, Mangalore.

Symptoms.—Cough with expectoration or asthmatic attacks were the presenting symptoms in all the cases examined. They were mostly between 21 and 40 years ago. Five cases gave a history of similar attacks in the other members of their family. A history of previous

attacks of eosinophilia treated with arsenic was elicited from 3 patients. Two others gave a previous history of cough. while 3 said that they had asthmatic attacks in the past.

Laboratory and other investigations.—I, Erythrocyte sedimentation rate was done by Westergren's method on 12 cases and showed a rise above 20 mm. per hour in 8 cases.

2. Kahn test was done on 8 cases ; 5 of them were negative.

3. Except for one case who was a diabetic and another who was about 70 years old and showed traces of albumin in the urine, all the other cases had a normal urine.

4. Sputum was negative for acid fast bacilli in all the cases examined.

5. Sternal puncture was done on 9 cases and was repeated to check progress on 3 cases ; in 2 cases it was repeated 5 times. The sternal puncture results showed in all the cases a proportionate rise in the eosinophils corresponding to a rise in the peripheral blood count. Bone-marrow study on further cases is still proceeding.

6. Microfilaria was not seen in the peripheral blood of any of these cases.

7. Percentage of eosinophils and total eosinophils. Blood for differential count was done almost daily in those patients admitted in the hospital, and as frequently as possible in those attending the out-patients' department. Total w.b.c. and total eosinophils by Hinklemann's method were also repeated, as frequently as possible, in all the cases. In all the cases of tropical pulmonary eosinophilia so far investigated, the eosinophil percentage was above 40 except in one case where it was 26 per cent. The absolute eosinophil count was above 3,000 cells per c.mm., except in one case where the count could not be done. The relationship between the total eosinophil count as calculated from the total w.b.c. count and eosinophil percentage and the absolute eosinophil count as found by Hinklemann's method has been compared. These counts were done at the same time on the same day by the same person. Out of a total of 35 periodic blood examinations, a difference of about 10 per cent between the two readings was noted in 17 findings.

8. *Biopsy.*—A female patient admitted with a high eosinophil count (62 per cent 7,875 total eosinophils) had a hard lump in the left breast. A biopsy of the lump was done and revealed a preponderance of eosinophils in the section. A diagnosis of eosinophilic granuloma of the breast was made. The patient was given a course of antimony (urea stibamine) to try the effect of antimony on eosinophils. With the treatment, the lump disappeared and there was a corresponding fall in the eosinophil count. Two other patients developed, one nodules and the other abscesses, when their eosinophil counts were still high. Biopsies were made to see if there was any infiltration of eosinophils in the nodules or in the wall of the abscess. A grouping of eosinophils in the nodules and wall cavity was reported by the pathologist.

9. Radiological examination was done on all the cases. Skiagrams were taken before the treatment was commenced when the eosinophil count was high, and subsequently at frequent intervals whenever possible. The result of this study is still to be analysed.

RELATIONSHIP BETWEEN EOSINOPHILS AND SYMPTOMS

The appearance in one case of eosinophilic granuloma of the breast and its relationship to the eosinophil count, and the development of nodules and abscesses in two cases have already been mentioned. It was found that even with a high eosinophil count many patients did not have any symptoms. In three cases it was noticed that they suddenly developed attacks of dyspnoea during the night or in the day time. Blood examination during the attack did not show any appreciable increase in eosinophil count. In such cases the effect of adrenaline injections on both the asthmatic attack and on the eosinophil count was noted. Surprisingly, although adrenaline had a temporary ameliorating effect on the asthmatic attack and relieved the dyspnoea, the eosinophil percentage both before and after the injection remained the same.

TREATMENT

Treatment of 17 cases of tropical pulmonary eosinophilia was conducted in the hospital—12 being admitted as in-patients and 5 as out-patients. Treatment was aimed at: (i) judging the effect of the various drugs available: (ii) the rapidity with which a fall in the eosinophil count could be obtained, and (iii) to judge if a combination of drugs could act better than a single one. The drugs used were arsenic by the oral (carbarsone) and/or: parenteral route (mapharside); hetrazan, modifications of hetrazan, e.g. Piperazine adipate (entacyl), cortisone (Delta cortil) and I.V. T.A.B. vaccine shock therapy.

The effect of the treatment can be judged from two points of view: (a) amelioration of symptoms, and (b) fall in the eosinophils. Whichever line of treatment was adopted, the patients said that they felt better. This probably was due to a psychological effect following the administration of a drug by the parenteral or oral route because in such cases there was not a corresponding fall in the eosinophils.

It was observed that hetrazan (piperazine dihydrogen citrate) given at a dose of 8 tablets per day for 14 days had the effect of bringing about a clinical improvement and caused a fall in the eosinophil count. This was tried in 8 out of the 17 cases. The fall of eosinophils percentage in these cases treated with hetrazan did not, however, go below 17 and total eosinophils in these cases did not fall below 1,540. With arsenic alone the 3 cases treated showed a fall to 15 per cent with 820 total eosinophils per cmm. The combination of arsenic and hetrazan was given to 2 cases with a fall in one case to 9 per cent and 1,085 total eosinophils. Piperazine adipate (entacyl) which was tried in 1 out of the above 17 cases and subsequently in another case not included in this series, did not show any effect on the eosinophils. Although it is premature to form any conclusion from just two cases, it may be

possible that the action of piperazine on the eosinophils takes place only after its absorption from the intestinal canal because entacyl is said to be not absorbed from the alimentary system.

SUMMARY AND CONCLUSIONS

1. Blood films taken from 100 healthy males and 10 females during two medical health check-ups in Mangalore have been examined to determine the average eosinophil percentage of the population of South Kanara. Forty-four one of the 100 males and 6 out of 10 females, showed a percentage of above 10.

2. Seventeen cases (12 males and 5 females) have been followed up at the Government Headquarters Hospital, Mangalore. Besides the usual laboratory tests, such as E.S.R., Kahn, etc., sternal puncture was done in 9 cases and repeated on 3 cases to check progress and in 2 cases it was repeated 5 times. A rise in the bone-marrow eosinophils is seen to be proportional to a rise in the peripheral eosinophil count.

3. A comparison is being made between the total eosinophils as determined by Hinklemann's method and eosinophils per cmm. as calculated from total w.b.c. and eosinophil percentage.

4. Biopsy study has been made from a case of eosinophilic granuloma of the breast of a patient who had a proportionate increase in the peripheral eosinophils. Two biopsies were made from eosinophilic patients who developed nodules and cutaneous abscesses. It was noticed that a concentration of eosinophils occurred at these lesions when the peripheral eosinophil count was high.

5. Series of skiagrams have been taken both before and after treatment.

6. The relationship between eosinophils and symptoms is discussed. A few eosinophilic patients suddenly developed, at times, asthmatic attacks with no change in their eosinophil count before and after the attack and also before or after treatment with adrenaline injections.

7. Treatment was varied from case to case. Different combinations are being tried. It is noted that whilst hetrazan had an effect in bringing down the count, entacyl (piperazine adipate) which belongs to the same group but is said not to be absorbed from the alimentary system had no effect.

42. Study of the antidiabetic activity of leaves of *Aegle marmelos* (Bili) under Dr. C.J. Modi and Dr. M.S. Kanvinde at the Seth Vadilal Sarabhai General Hospital, Ahmedabad.

In the last 1½ months for which this inquiry has been running, 3 patients have been selected for *Aegle Marmelos* therapy. Since the maximum period of investigation has been six weeks, follow-up glucose-tolerance tests have yet to be carried out. Under the circumstances, it is considered to be too early to draw any conclusions.

43. Investigations into the factors affecting the susceptibility of diabetes to infection under Dr. T.P. Bharadwaj at the Topiwala National Medical College, Bombay.

Experimental.—The diabetes was experimentally induced in albino rats by alloxan. The mortality was about 80 per cent in younger rats as compared with rats weighing about 150 g. to 180 g. In the latter, with the alloxan given in doses of 180 mg./kg. of body-weight intraperitoneally, mortality was 40 to 50 per cent. One to two units of insulin had to be given in rats showing hyperglycæmia with acidosis. The latter was judged by the presence of acetone bodies in urine.

In 40 normal as well as diabetic albino rats the following were determined : Blood eosinophil count, 17 ketosteroids and hydroxysteroids of urine, urinary uric acid and creatinine. The results are as under :—

No.	Investigations	Values in normal rats.	Value in diabetic rats.
(i)	Blood eosinophils/cmm.	20—400	0—60
	24-hour urinary excretion of :		
(ii)	17 ketosteroids	0.06—0.15mg.	0.135—0.5 mg.
(iii)	Hydroxysteroids	14—35 ug	80—135 ug.
(iv)	Creatinine	1—3.5 mg.	0.175—0.66 mg.
(v)	Uric acid	0.18—0.75 mg.	0.1 —1.5 mg.

The eosinophil count was very much reduced in early acute toxic phase of the diabetes. It was found that uric-acid excretion did not follow a set pattern. The urinary sugar was 1 to 3 per cent in all diabetic rats.

Repeated blood-sugar estimations or estimations of plasma proteins was not undertaken since bleeding could cause sufficient stress and thus vitiate the results.

Granuloma pouch with I.c.c., 1 per cent croton oil was produced in normal as well as in diabetic rats by the technique described by Salye. The rats were sacrificed and the granuloma pouch examined from 3 to 10 days of rats inception. It was observed that both naked eye as well as histologically inflammatory responses were very much inhibited in diabetic rats. The zone of necrosis was distinctly increased in the diabetes. Histochemical studies showed less marked depolymerization of the ground substances during inflammation. The antiphlogistic phase was more markedly evident in acute toxic phase of the alloxan diabetes.

The pancreatic lesions in the diabetic rats were confirmed post-mortem by employing Gomori's-phloxine chrome-haematoxyline method. Adrenal hyperactivity in diabetic rats has been confirmed by histology. Excretion of creatinine was found to be increased in later phases of diabetic rats with granuloma pouch, viz. of the order of 2 to 3 mg. day. These rats were also having high rate of 17 keto and hydroxysteroid excretion and low eosinophil count. Creatinine excretion perhaps did not serve as a faithful guide of hormonal levels in them. Probably, this could be due to this chemical being liberated from necrotic muscles mass.

Human.—Following hormonal assays have been undertaken in human beings (10 normals and 10 diabetic patients).

Number	Steroids	Normal.	Diabetic
1.	24-hour total 17 ketosteroid excretion	6—18 mg.	3.3—16.8 mg.
2.	„ „ 17-OH steroid	0.5—1 μ gm.	1.1— 4.8 μ g.

Thus, diabetes show a distinct evidence of adrenal hyperactivity.

Values have been determined for 17-hydroxysteroids in 56 normal blood samples.

This gives a range of 45 μ g to 85 μ g. with an average of 53.56 ± 16.95 μ g. per 100 c.c. of blood.

44. Biochemical studies on tumor under Dr. S.C. Roy in the Department of Applied Chemistry, Calcutta University, Calcutta.

Warburg first showed that tumors possess a high rate of anærobic glycolysis. Human tissue under different conditions of growth—normal, fibroid or malignant—was, therefore, analysed with respect to the intermediates of the glycolytic cycle. For the purpose, malignant tissue from human uterus, and some fibroid—and normal analogous tissues from different patients in various hospitals of Calcutta were taken just after the surgical removal and carried under ice-cold condition and in some cases under liquid air and worked up for :

- (a) Glycolytic phosphate esters
- (b) Free and total amino acids
- (c) *In vitro* utilization of C^{14} from labelled acetate by a fibroid tissue.
- (d) Krebs cycle α -keto acids

(e) Nucleic acids

(a) *Glycolytic phosphate esters*.—Study of the glycolytic intermediates indicates that organic phosphorus increases as the tissue changes from normal to malignant condition, while the distribution of inorganic phosphorus keeps on more or less the same value. The highest increase is observed in the levels of hexose and triose-phosphates under fibroid or malignant condition. This increased formation of phosphate esters might be due to the increased glycolysis in tumor. Consequently, phosphorus is collected more by the tumor tissue, and this perhaps provides some experimental basis for the therapeutic use of P^{32} in neoplastic disease.

(b) *Free and total amino acids*.—Any vital change in protein associated with malignancy might well be reflected in changes in their amino-acid composition. It was, therefore, of interest to determine quantitatively by the chromatographic method the free and total amino acids in different human tissues from the above cases and conditions. Seventeen amino acids have been identified and quantitatively estimated. From the results obtained uptil now, though it cannot be said that a shift in the amino-acid composition in any particular direction is characteristic of malignancy, but it was observed that the concentration of some of the amino acids in the tissue hydrolyzate, particularly alanine, aspartic acid, and glutamic acid, are significantly lower under malignant condition. In free form the concentration of these amino acids was, however, higher in malignancy. This seems to indicate that certain amino acids are preferentially mobilized and metabolized for neoplastic purposes.

(c) *In vitro utilization of C^{14} from labelled acetate by a fibroid tissue*.—Results obtained in (a) and (b) give some indications as to the preferential utilization of particular compounds in the metabolism of tumor tissue. Some exploratory work with acetate— $1-C^{14}$ has been carried out to study the uptake of C^{14} by different amino acids present in the tumor tissue with one typical experiment. Incorporation of C^{14} has been observed in the case of alanine, phenylalanine, leucine, valine, serine, glutamic acid, lysine and aspartic acid.

(d) *Krebs cycle α -keto acids*.—Since tumor is an uncontrolled anabolic process, certain metabolic peculiarity is expected in the case of neoplasm, particularly with respect of Krebs cycle intermediates. In the present investigation it has been observed that the concentrations of alanine, aspartic and glutamic acids are lower in fibroid and lowest in malignant tissues. These three amino acids on oxidative deamination are converted respectively to pyruvic, oxaloacetic and α -ketoglutaric acids which are the key keto acids of Krebs cycle. The determination of these acids in some cases of normal, fibroid and malignant tissues has been carried out.

(e) *Nucleic acids*.—Since nucleic acids are the important biochemical constituents of the cell, it was of interest to determine the nucleic-acid contents of the tissue under normal, fibroid or malignant conditions. The determination is based on PNA-P and DNA-P (Pentose or deoxyribose nucleic acid phosphorus). Some preliminary work has been done in this line.

45. Inquiry on the effects of irradiation on hæmopoietic system in patients suffering from malignant diseases under Dr. B. Basu and Dr. Hari Sadhan Dutta at the Chittaranjan Cancer Hospital, Calcutta.

The plan is to study the effects from different aspects, e.g. (i) hæmogram, (ii) myelogram, (iii) bio-chemical analysis, and (iv) gastric content analysis. To start with, the study on hæmogram has been taken up.

Histopathologically proved malignant cases are being taken up, avoiding weak, anæmic and advanced group of patients. The first series of enquiry consists of male cases restricted to neck and face regions, e.g., cancer tonsils, cancer larynx, cancer antrum, cancer cheek, cancer neck, lymphosarcoma neck glands, etc.

The hæmatological examinations are being performed according to the standard methods mostly adopted in the School of Tropical Medicine, Calcutta. In all cases the pre-radiation readings are recorded and followed by weekly variations in post-radiation period.

In the study of hæmogram the following are taken up : (1) hæmoglobin in grammes (Sahli's method), (2) total counts in red and white blood corpuscles, (3) differential counts in white blood corpuscles, (4) reticulocyte counts, (5) thrombocyte counts, (6) erythrocyte sedimentation rate, (7) mean corpuscular volume, (8) mean corpuscular hæmoglobin, and (9) mean corpuscular hæmoglobin concentration.

Patients were irradiated either from million-volt or 200 to 250 K.V. machines. The dose was calculated in the following ways : (1) Air dose in roentgen, (2) skin dose calculated with back scatters which is constant for each individual machine. (3) tumour dose, (4) integral factors—in each case worked out graphically, and (5) integral dose, calculated from skin dose.

Experimental Data.—The available data obtained within a short period of three months' work reveals the following :—

1. The effect of irradiation on hæmogram by million-volt machine has been studied in three cases only. The lymphocytic depression was prominent in all the cases. The effect on the other corpuscular element is not very significant.

2. The effect of irradiation on hæmogram by 200 to 250 K.V. machines was studied in 12 cases. The data so far obtained show that in majority of the cases there is depression in total leucocyte and lymphocyte counts. In the case of 1, lymphosarcoma neck glands there is increase in total leucocyte and neutrophil counts during the post-irradiation period with progressive fall in lymphocyte counts, e.g., pre-radiation : neutro-2,520; lympho-2,310; post-radiation : neutro-6,090; lympho-725.

3. The effects of irradiation on thrombocytes are observed to have a rise in some cases by 200 to 250 K.V. therapy, but with million-volt therapy the value is almost constant in two cases and rises in one.

4. Determination of E.S.R. in all the cases shows that the readings are high in the pre-radiation period. In case of lympho-sarcoma neck glands the value was low. It is observed that with the irradiation the rate of sedimentation per hour increases except in two tonsillar cases and one epiglottis case where there is a tendency to fall in the rate of sedimentation rate but the reading remaining at a higher level than normal even at the end of irradiation therapy.

46. Clinical study of neuropathies under Dr. P.N. Chuttani at the Medical College, Patiala.

Thirty-four cases of peripheral neuropathies have been investigated and these consisted of the examples of diabetic neuropathy, Landry-Guillain-Barre syndrome, sub-acute combined degeneration of the cord, idiopathic polyneuritis, alcoholic polyneuritis, arsenical polyneuritis and post-dysenteric polyneuritis. A detailed *proforma* was filled in each case and complete investigations, including a peripheral nerve biopsy, were done. Old discharged patients of neuropathies seen before the present study were also followed up.

A total of 35 peripheral nerve biopsy specimens was obtained : one inch of nerve was taken from the medial side of the second toe and put in two different fixatives, consisting of (a) neutral formalin 10 per cent, and (b) osmic acid 2 per cent. Frozen sections of a few specimens fixed in neutral formalin were cut and examined by the Polarization method. Some neutral formalin specimens, after their blocks were made, were stained by Masson's trichrome method and in others Azocarmine staining (Heidenhain's modification of Mallory aniline blue) was done to show minute details of the connective tissue. The osmic acid specimens were studied for the myelin sheath. Frozen sections were also stained for the myelin sheath and axis cylinder.

47. Inquiry on the role of *Entamoeba coli* as a pathogenic amoeba under Dr. R.M. Kasliwal at the S.M.S. Medical College, Jaipur.

Among the intestinal protozoa found in man only *Entamoeba histolytica* has been labelled as pathogenic and the rest including *Entamoeba coli* as non-pathogenic. Quite contrary to the popular belief we have seen a number of cases clinically presenting a picture of amoebic syndrome, but where only *E. coli* were found in stools on repeated examinations and no *E. histolytica* were demonstrated. In three cases we were successful in demonstrating ulcers in rectum and sigmoid colon on sigmoidoscopic examination. In two cases the scrapplings from these ulcers showed *E. coli* organism and no *E. histolytica*. These cases of pure *E. coli* infection responded well to the anti-amoebic line of treatment. We believe that *E. coli* is also a pathogenic organism producing a clinical picture akin to *E. histolytica* infection. It is possible that virulence of *E. coli* may be less than *E. histolytica*. The aim of this enquiry is to follow this problem for evaluation. Some work has been done in this direction in foreign countries, though the reports from

different sources are not complete. Experiments of Rendtroff indicate that infection of a human volunteer occurred following in ingestion of a single cyst of *E. coli*. Rockman and Epstein in their paper entitled 'Clinical symptoms associated with the so-called non-pathogenic amœbæ' have made the observation that all amœbæ occurring in the intestine are potentially pathogenic. Brumpt has reported that inoculation of a cat with cysts of *E. coli* and *Entamœba* in association, from a human case, resulted in formation of small intestinal ulcers containing amoebæ. The findings suggest that *E. coli* under certain conditions might become pathogenic, since in his hands, *Entamœba dispar* does not give rise to intestinal lesions in cats. Evidences are also available that *E. coli* are capable of ingesting red blood cells both in culture (Dobell, 1938) as well as in intestine (Tyzzer and Geiman, 1938).

Our procedure of collecting these cases of pure *E. coli* infection is : A thorough history is taken and physical examination with special reference to gastro-intestinal tract is done. Blood (total and differential leucocyte count, total red cell count, hæmoglobin and E.S.R.) and urine, are examined as a routine. Special attention is paid to stool examination. The stool is examined every day for 3 to 4 days according to the following plan :—

- A. Macroscopic examination.
- B. Microscopic examination.
 - (i) Direct fæcal smear.
 - (a) In normal saline.
 - (b) In Lugol's iodine.
 - (c) After staining with iron hæmatoxylin stain.
 - (ii) After zinc sulphate-floatation concentration.

In addition to this, positive stools are also cultured on Boeck and Drbohlav's Locke—egg-serum (L.E.S.) medium, and Craig's normal saline serum medium. Sigmoidoscopic examination is also done with the aim of detecting any lesions and demonstration of the causative organism in the lesion.

After finding pure *E. coli* infection these cases are given an anti-amoebic course of treatment and the therapeutic response is recorded. Anti-amoebic course of treatment given is : Injecting procaine penicillin 4 lac. units i.m. daily for 3 days followed by injection emetin grain I. i.m. daily for 4 days. Emetin is followed by carbarsone one tablet, twice daily, for 10 days. In addition, specific treatment, vit. B. complex and liver extract is also given as it is observed that most of the patients also suffer from some amount of malnutrition and vitamin B—complex deficiency.

We have uptil now observed 27 cases with clinical amoebic syndromes but only *E. coli* infection and not *E. histolytica* infection. Out

of these. 23 cases were given complete anti-amoebic treatment—18 of these cases got relieved, and the remaining 5 cases had partial relief. These five cases were advised to take second course after a month. Stool examination after completion of the course showed that 14 cases were free of *E. coli* 4 cases left against medical advice 3 after partial, and one without any treatment. Sigmoidoscopic examination was done in 8 cases and ulcers resembling ulcers of *E. histolytica* infection were demonstrated in 3 cases. In two of these cases trophozoites of *E. coli* were demonstrated microscopically in the material obtained from scrapings of the ulcers. Stool culture was done in eight cases and pure *E. coli* growth was obtained in three cases. In the rest 5 cases, no protozoal growth could be obtained. The failure in getting positive cultures in these five cases is perhaps attributable to the fact that the culture work has only recently been started by us and the technique has not yet been mastered.

48. Inquiry to investigate the mechanism of antibacterial action of specific antiserum under Dr. J. C. Ray at the Indian Institute for Medical Research, Calcutta.

The scheme was submitted to follow up the clue indicated by the work in the institute initiated by Drs. B.R. Chatterje and S.N. Chaudhuri under the guidance of Dr. J.C. Ray. Some preliminary work was done on the kinetics of the adaptive enzyme, β -Galactosidase in *E. coli* using a simpler modification of Thunberg's methylene blue reduction technique. The workability of this method was compared with the Thunberg's technique which showed a high degree of statistical correlation, proving this method almost of the same working efficiency as the Thunberg's. As to the effect of the anti-serum on the bacterial metabolism, it was shown that the anti-serum could delay the synthesis of the adaptive enzyme, β -Galactosidase, considerably when a lactose de-adapted strain of *E. coli* was exposed to it. The lactose adapted strain would not, however, be affected similarly. This effect again was observed only in a zone of limited inducer substrate concentration, an excess supply of the inducer substrate would not allow the anti-serum to manifest its effect. This anti-adaptive effect of anti-serum could be counteracted with the addition of a little amount of ATP (3×10^{-5} M/c.c.).

The work done, so far, has been channellized into two directions, namely, (i) the effect of ATP on the adaptation of the de-adapted strain, and (ii) utilization of lactose from the medium by the lactose adapted (L) and de-adapted (X) strains. The data obtained are detailed below :

The effect of ATP.—Synthesis of enzymes being an energy activated process, it was thought that ATP, the energy rich substance, could have some effect on the adaptation of the X strain and it is, and after re-exposure to anti-serum. As such, experiments were done with ATP, added to the X strain before and exposure to anti-serum, along with or without lactose. It was observed that ATP, when added to the X strain as such, in a concentration of 3×10^{-5} or 3×10^{-6} M/c.c., along with

lactose $0.2 \mu\text{m}/\text{c.c.}$, could bring about a much quicker adaptation compared to that with lactose or ATP alone, in the same concentration.

	TUBE NUMBERS						
	1	2	3	4	5	6	7
Bacteria	+	+	+	+	+	+	+
Lactose	+	+	—	+	—	+	—
ATP $3 \times 10^{-5}\text{M}/\text{c.c.}$	—	+	+	—	—	—	—
ATP $3 \times 11^{-6}\text{M}/\text{c.c.}$	—	—	—	+	+	—	—
ATP $3 \times 10^{-7}\text{M}/\text{c.c.}$	—	—	—	—	—	+	+
50 per cent dye reduction in minutes	86	72	106	78	120	88	120

ATP in a concentration of $3 \times 10^{-5}\text{M}/\text{cc.}$ along with lactose, effect adaptation of the anti-serum exposed X-strain almost in the same period as with normal serum.

	TUBES NUMBERS			
	1	2	3	4
Bacteria	+	+	+	+
Normal	+	+	—	—
Antiserum	—	—	+	+
Lactose	+	+	+	+
ATP $3 \times 10^{-5}\text{M}/\text{c.c.}$	—	+	—	+
50 per cent. dye reduction in minutes				
Pre-incubated with lactose	63	45	80	52
Not pre-incubated	88	70	110	73

Whether the ATP effect is due to the liberation of high energy from the phosphate-bonds or due to the utilization of the ribose molecule in it, as substrate has not yet been clearly elucidated. Further studies are being continued in this direction by employing AMP, adenosine and adenine.

Lactose utilization from the medium.—If the effect of anti-serum on the adaptive functions was operative in the level of cell permeability to the substrate, it could probably be explored by determining the rate of lactose utilization by the X-strain as such and after exposure anti-serum. The results of these experiments are detailed below.

Utilization of lactose by the L and X strain.—As will be evident from the table below, while 95 per cent of lactose was used by the

L-strain in one hour's time, the X-strain failed to utilize at all during the same period.

Time in minutes :	Per cent lactose utilized			
	15	30	45	60
L-strain	50	80	9	95
X-strain	Nil	Nil	Nil	Nil

Utilization of the lactose by the X-strain after exposure to anti-serum.

—Very little amount of lactose is utilized by the anti-serum exposed X-strain if it is not preincubated with lactose beforehand. The cells exposed to normal serum, however, could utilize almost half of the lactose in 1 hour even if it is not pre-incubated with lactose.

Per cent lactose utilized by the X-strain after exposure to anti-serum and normal serum		
	Pre-incubated with lactose	Not pre-incubated
Normal serum	90	46
Anti-serum	45	14

49. Inquiry on electron-microscopic investigations at the Institute of Nuclear Physics, Calcutta.

Electron microscopic investigations have been carried out on the cytology of the flagellate form of *Leishmania donovani*. both by the technique of ultra thin sectioning and the disruption technique. The periplast or the pellicle of the micro-organism has been found to be a thin structureless layer, about $70 \text{ \AA}^\circ$ ($7 \text{ m } \mu$) thick. On its inner side there are numerous subpellicular fibrils, running more or less parallel to each other and following a spiral course around the body of the protozoan. Their mean diameter is $250 \text{ \AA}^\circ$ ($25 \text{ m } \mu$) while the average separation between two fibrils is $200 \text{ \AA}^\circ$ ($20 \text{ m } \mu$). These fibrils show a regular periodicity along their length, which may be due to an actual periodicity in their structure or may be due to the presence of still finer sub-fibrils spirally twisted around each other. They have never been found to be branching and it is concluded that the ramification described by some previous authors in these fibrils were artifacts produced by faulty methods of preparation.

The flagella of the parasite has been found to be about 0.15μ to 0.2μ in diameter uniformly along its whole length. The tip is always rounded and never tapering. The flagella has been found to be provided with a sheath about $70 \text{ \AA}^\circ$ ($7 \text{ m } \mu$) thick, which is of the same order as the pellicle over the body; probably the flagellar sheath is a continuation of the body pellicle.

B. MYCOBACTERIUM LEPRÆ

Preliminary investigations on the structure of *M. Lepræ* have been started, with particular emphasis on the effect of various methods of

extraction and other treatment on the bacillus. It has been observed that osmic acid vapour fixes the micro-organism sufficiently well to enable one to study the details of the structure. Treatment of the bacillus with distilled water, and extraction with chloroform cause disruption of the protoplasm of the organism preserving only the cell wall outline. Using the first mentioned process, the various stages of the life cycle of the bacillus have been studied under the electron microscope.

50. Study of the bacterial flora of the skin in cases of dermatomycosis under Prof. R.K. Gadgil at the B.J. Medical College, Poona.

Abstract.—Twenty-five cases suffering from various mycotic infections were selected from the out-door patients of the Sassoon Hospitals, in consultation with the Hony. Skin Specialist. The cases consisted of various mycotic diseases of the skin. From the skin lesions material was collected for isolation and study of the bacterialflora and skin scrapings were collected for isolation of the fungus.

The primary bacterial cultures were done in broth medium and organisms identified on sub-cultures and other confirmatory tests. The bacterialflora isolated so far show the presence of the following organisms: *Staphylococcus albus*, *Staphylococcus citreus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus viridulus*, *Proteus retgeri*, *Micrococcus cinnabareus*, *Streptococcus fecalis*, *Neissaria sicca*, *Micrococcus freudenrenchi*, *Micrococcus luteus*, *Bacillus pyocyaneus*.

The fungus cultures were done on Saboraud's medium and are under study. No conclusions have been drawn as the number of cases studied so far is insignificant.

51. Studies on metabolism of the catechol amines in bronchial asthma and tropical eosinophilia under Dr. H.S. Chakravarti at the Indian Institute for Medical Research, Calcutta.

Uptil now, 9 cases of tropical eosinophilia and 4 cases of bronchial asthma have been investigated. As control, 11 normal cases, selected from the laboratory workers were also investigated.

Besides through clinical examination, following investigations were carried out :

(1) Twenty-four hours urinary excretion of catechol amines were estimated following the method of u.s. Von Euler (*Acta Physiolo Scand.*, 1955, **33**, Suppl. 118). Urine was collected for 24 hours with 5 c.c. concentrated Hcl as preservative and stored in refrigerator before estimation. Amines were absorbed on aluminium oxide and eluted with H_2SO_4 . Final estimation was done on spinal cat, recording the change in blood pressure and comparing with standard adrenaline hydrochloride solution.

(2) *Twenty-four-hour excretion of 17-ketosteroid and ketogenic steroid.*—Estimation was done by the method of Callow (Hormone Assay, Academic Press, New York, 1950).

(3) Thorn's test were performed in a few cases using 20 units of ACTH (Dumex).

RESULTS

Catechol amines excretion in 24 hours urine :

Normal.—In 11 cases of normal adults, including 7 males and 4 females. The result was within the range of 11.5 and 63.4 microgramme equivalent of adrenaline with the mean value of 38.5 microgramme.

Tropical eosinophilia.—In 9 cases of tropical eosinophilia all adults, 3 females, rest male, before instituting the arsenical treatment, the result was within the range of 15.5 to 70.9 microgramme equivalent of adrenaline, with a mean value of 37.1 microgramme. After recovery with arsenical therapy, the range was 15.8 to 63.8 microgramme in five cases, followed up to the date, with mean value of 47.0.

Bronchial asthma.—In four adult cases, 2 female and 2 male the results were 27.7, 99.0, 59.5 and nil microgrammes. In one case value obtained was slightly higher than normal. In the another case, there was reverse action on blood pressure which could not be countered even with anti-histaminics in spinal cat. Nature of the depressor material is being investigated.

Number of cases are too small for any statistical analysis. However, it appears that there may not be any appreciable variation from normal values in the quantity of catechol amine excreted in the urine in the case of Tropical Eosinophilia, before and after therapy.

17-ketosteroid excretion in 24 hours urine ;

Normal.—In 6 cases of normal male adult the range was 6.5 mg. to 10.4 mg. with a mean of 8.4 mg.

Tropical Eosinophilia.—In 6 male patients five were adult and one was adolescent. In the five cases the results were in the range of 5.1 mg. to 8.8 mg. with a mean value of 6.6 mg. The boy excreted out 1.9 mg. In the three females the results were 1.5 mg., 2.5 mg., and 2.2 mg.

In 3 adult males 17-ketosteroid were estimated after recovery with arsenic therapy. All of them showed slight increase in the value.

Bronchial asthma.—In two males the results were 5.4 mg. and 6.8 mg. and in two females 4.1 mg. and 5.3 mg.

Thorn's test.—This test was performed in 4 cases of tropical eosinophilia using 20 units of ACTH (Dumex). Initial total eosinophilic counts were 7,150, 23,700, 17,150 and 8,894, per cu.mm. of blood. After 4 hours of injection of ACTH, the counts came down to 4,368, 23,100, 9,700 and 4,300 indicating a reduction of 38, 3, 44 and 51 per cent., respectively. Except in one case, which unfortunately we failed to follow up, all showed appreciable reduction of eosinophilic count after ACTH injection.

52. Inquiry on the relative values of rest and movements in the treatment of intra-articular fractures—an experimental study under Dr. B. Mukopadhyaya at the P.W. Medical College, Patna.

With a view to study the effects of the two methods of treatment in intra-articular fractures, namely, by early movement and by plaster immobilization, operations were performed in a series of goats producing intra-articular fractures of varying degrees of severity ranging from a simple intra-articular crack to a severe comminuted fracture. Goats were operated in groups of two, in one the fractured joint was plastered for 4 weeks, while in the other the operated joint was left free except for a light dressing for the wound. Both the animals were permitted to use their limbs while walking, in the first, the limb being in plaster cast, while in the second, the limb was without any such method of immobilization.

Their respective rates in the recovery of joint function was noted.

The results of experiments so far performed are as follows :—

- (a) In every case of joint fracture where no post-operative immobilization was used and the animal was encouraged to move the limb right from the date of operation, joint function returned to normal within 3 to 4 weeks after the operation with full and complete restoration of movements as compared to the normal side. The animals used the limbs freely.
- (b) In every case where the joint was immobilized in plaster of Paris spica following operation, the joint was found to be completely stiff on the removal of plaster at the end of 4 weeks of plaster immobilization. Following removal of plaster, recovery of movements at the joint has been extremely slow and gradual and full range of movements have not returned even 2 months after removal of plaster. None of the animals in this group are able to use their limbs normally.

Thus, from this short series of experiments it appears that early mobilization causes much earlier and much better restoration of joint function in cases of intra-articular fractures than prolonged immobilization following the fracture. A much larger series of experiments involving many different joints must, however, be performed before any definite and final conclusion can be drawn. The results so far obtained, however, are very suggestive, specially in view of the uniformity of these results.

In order to obviate the fallacy that mere immobilization of a limb without any articular damage may cause joint stiffness, a series of animals were immobilized in plaster for periods varying from 2 to 6 months. None of these animals developed any persistent joint stiffness and following removal of plaster, joint movements returned to normal range within 2 weeks.

CHOLERA

1. Inquiry on the state of anuria and uræmia in cholera with special reference to its treatment under Dr. A Das at the Nilratan Sircar Medical College, Calcutta.

Thirty-one cases of uræmia in cholera were investigated 30 cases developed uræmia during treatment ; one was admitted in the state of uræmia.

In addition to a detailed clinical survey, laboratory investigations were done to estimate the state of extracellular and intracellular hydration. Plasma concentration of Sodium, Potassium, Calcium, Chloride, and Phosphate were estimated in 25 cases, blood Urea in all and plasma bicarbonate in 28 cases. Electrocardiographic studies were done in 15 cases.

Intracellular overhydration was present in 11 cases and 8 of them were unconscious or semiconscious. Hyponatræmia was present in 15 cases and manifest hypopotassæmia in 6. The latter was associated with diminished peristaltic movement and abdominal distension of varying degree, and diminution of deep reflexes. Only one case died of terminal hyperpotassæmia.

All the cases were associated with a state of acidosis which was of severe degree in 13. The increase in the depth of respiratory excursions ran parallel with the degree of acidosis in all except one.

Three types of biochemical changes were associated with the acidotic state (i) Low Plasma Sodium—15 cases (ii) High Plasma chloride—5 cases (iii) Without Hyponatræmia or Hyperchloræmia—5 cases.

Electrocardiograms were taken in 15 cases of which 6 died. Tracing suggestive of hypopotassæmia were present in 6 cases : 2 of these died. One case died of cardiac arrest from hyperpotassæmia in the terminal stage.

The mortality rate of 16 patients treated on principles suggested by Byll (adapted to cholera) was 25 %. 15 cases treated with standard alkaline saline had a mortality rate of 53 %.

2. Cholera inquiry under Dr. K. Bhaskaran at the Central Drug Research Institute, Lucknow.

In a collection of 127 strains of *V. Cholerae*. 30 strains were found to be lysogenic. The associated temperate phages could be demonstrated by the appearance of plaques when spotted on sensitive indicator strains.

Among the lysogenic strains were representatives of both the sub-types Inaba and Ogawa of *V. cholerae* (O group I, Gardner and Venkatraman). The exact differentiation of the various phages is not yet complete, but it appears probable that there may be quite a few types among them. This is of interest in view of the observation of Bruce White (1937) that all Indian strains of *V. Cholerae* were infected with LL phage, which would show up only when tested on Japanese strains. The success of cross-matching tests, which has now resulted in the identification of several lysogenic strains within an Indian collection, show that there may be phages other than LL, genetically associated with *V. Cholerae*.

2. *Genetic Studies*.—Attempts were made to explore the possibility of genetic transduction in *V. Cholerae*. This phenomenon involves the transfer of a 'marked' character from one strain to another through the agency of temperate phage, and has so far been demonstrated only in *Salmonella* species. With lysogenisation of sensitive strains, which these phages are capable of, some of the genetically controlled properties of the donor strain are also known to be transferred (transduced) to a small proportion of the lysogenised recipient cells.

The availability of several lysogenic strains made such studies possible in *V. Cholerae*. The property of Streptomycin resistance *i.e.* the ability to grow in the presence of 500 units of streptomycin sulphate per ml. of medium, was utilized as a potential transducible character. The technique employed was to seed Streptomycin sensitive indicator strains, in high titre phage lysates of a Streptomycin resistant mutant of INABA 52, obtained with various lysogenic strains. After varying periods of incubation at 37°C, these cultures were plated on Streptomycin Agar, along with controls grown in nutrient broth. The isolation of a significantly larger number of Streptomycin resistant colonies from phage preparations, in contrast to controls would indicate transduction.

Under the conditions employed, transduction of Streptomycin resistance could not be demonstrated with 16 lysogenic strains of *V. Cholerae* so far studied. Further work is in progress with the other lysogenic strains and attempts are also being made to transduce other characters such as 'nutritional markers', motility and 'O' antigenic factors.

3. *Nutritional Studies*.—Based on the studies of Bhaskaran and Rowley (1956), who showed that a large number of strains of *V. Cholerae* required purines for growth, attempts were made to devise a simple medium for the growth of *V. Cholerae* comprising only the essential nutritive materials. Such a medium would be useful for the laboratory cultivation of the organism, as well as for its isolation from infected materials. This work is in progress, but so far it has been found that a medium comprising 6 hydroxy purine (hypoxanthine) 0.1 per cent, Methionine 0.1 per cent. glucose 0.0 per cent. Sodium Chloride 0.5 per cent. and ammonium sulphate 0.1 per cent. buffered to pH 8.0

was adequate for the growth of several strains of *V. Cholerae*. The test strains included those which were non-exacting and capable of growing on ammonium sulphate and glucose as sole sources of N. and C. a strain which required methionine for growth, strains which required purines for growth, strains requiring purine and methionine for growth. The only draw-back so far observed with this medium was the smaller colony size of the organism, compared to other complex protein digest media. It is hoped that with further supplements, the nature of which have still to be elucidated, the medium could be improved and rendered as good as any other employed for the cultivation of this organism.

3. Inquiry on the evaluation of the 'phages acting on vibrios and the application of bacteriophage typing in epidemiological investigation on cholera under Dr. M.N. Lahiri and Dr. B. Ghosh Roy at the All-India Institute of Hygiene & Public Health, Calcutta.

Object.—This study has been undertaken to find out the possibility of typing strains of *V. cholerae* with the help of different cholera phages on the basis of the lytic pattern and to evolve a phage typing scheme to trace the source of infection and relationship between cases. The phages will also be used to elucidate the inter-relationship between the *V. cholerae* and other NAG vibrios isolated from the stools of cholera cases or from other sources.

Synopsis of work done.—During the last cholera season of this year, further isolations of strains of *V. cholerae*, the associated NAG vibrios (in some cases) and phages were made from the stools of patients. As reported previously, the simultaneous use of Wilson and Blair's enrichment medium (liquid) and bile-salt agar-plates (pH of both media ranging between 8.4 and 8.6) significantly improved the percentage of isolations. In contrast to the previous year, where all the strains isolated were of Inaba subtype, the majority of strains (over 85 per cent isolated this year were of Ogawa subtype).

Preliminary standardization of media for the isolation of phages from stool samples indicated the usefulness of alkaline broth. The phage plaques were best demonstrated on freshly prepared 1.2 to 1.5 per cent nutrient agar (Difco). The use of above medium not only improved the chances of their primary isolation but also facilitated the study of the lytic patterns. Of the phages isolated this year, 12 were from Ogawa positive stool samples, and 3 from Inaba positive ones. They have now been obtained as 'pure' line strains.

Propagation in liquid media enhanced the titre of some of the phage preparations although the same did not hold true for all the strains. The phages, isolated from stool filtrates, are being maintained by propagating on the corresponding strain of *V. cholerae* isolated from the same sample of stool. Storage of these phages at 0 C to 4 C showed

variation in the rate of fall in titre, thereby necessitating frequent transfers in the case of certain phage strains.

The yield of phage in association with growing culture of susceptible strain on semi-solid agar was investigated. The results obtained so far indicate the possibility of obtaining phage preparations with augmentation of titre, in spite of the fact that the method is more complicated than the routine procedures. The other susceptible strains of vibrio are being employed for enhancing the virulence of the phages.

The lytic picture of a few 'pure' line phages has been studied. The discrete plaques may vary in their size, viz., big, medium, small and in some cases even pin points. A distinct halo around the plaque was also evident in some cases. The significance of these findings is being investigated. Phages isolated originally from Inaba positive cases when titrated (undiluted) against different Inaba subtype strains showed certain differences in action. Certain strains were found to be completely resistant to the lytic action of certain races of phages but the same phage preparations, when tested on other strains of the Inaba subtype, showed complete clearing or a few discrete plaques. Since some of phages have also been found to be active against Ogawa subtype strains and vice versa, a start has been made to determine the 'critical test concentration' for every phage preparation against the homologous vibrio strain. It is possible that the use of phages at optimal dilution may show more significant differences in the lytic patterns.

In view of the recent trend that certain bacterial species and types owe their characters to the latent phage or symbiotic phage they carry (lysogenic strains), the stock strains of Inaba and Ogawa subtypes are being cross-titrated for their resistance to the lytic effect of the phage preparations in hand. These resistant strains will be studied further and used for adaptation experiments.

4. Cholera inquiry at the Central Research Institute, Kasauli.

The immunological studies on the vibrios were started with a study of the immunological and chemical properties of the polysaccharides obtained by the method of Shrivastava *et al* (*Ind. Jour. Med. Res.*, 1948, **36**, 402) and by the method of Linton (*Ind. Jour. Med. Res.*, 1932, **20**, 347).

The polysaccharides were prepared from Inaba 53/54 by (i) extraction with phenol and (ii) extraction with acid. The two preparations were studied for their immunological, physical and chemical properties. The polysaccharide obtained by extraction with phenol had a total nitrogen content of about 7 to 14 per cent, total phosphorus 3.3. to 4.5 per cent, reducing substances 20 to 25 per cent and viscosity 1.1 to 1.3. It gave precipitation reaction with homologous serum, protected mice against lethal doses of *V. Cholerae* and evoked agglutinins in rabbits and produced bactericidal response in guinea-pigs. The polysaccharide

obtained by extraction with acid evoked agglutinins in rabbits and did not have protective action for mice. The preparation in both cases seemed to be an antigenic complex. Acid treatment of the polysaccharide obtained by phenol extraction gave a soluble, an insoluble, and an ether soluble fraction. The soluble portion had immunological properties similar to those of the preparation obtained by acid treatment of the bacteria.

The residue from the acid extraction of the bacteria was further extracted with phenol and gave a polysaccharide preparation with about the same immunological and chemical properties as that obtained by straight phenol extraction.

The chemical composition, with regard to the sugar constituents, of the polysaccharide preparations from Inaba strains 53/54, 569B and a non-agglutinable strain W.G., obtained by phenol extraction, showed that they contained a pentose (most probably ribose), glucose and/or glucuronic acid, galactose and a hexosamine. The presence of ribose is a very interesting observation as it shows that the vibrio polysaccharide contains a pentose different from that of arabinose, stated by Linton *et al* to be present in some of the vibrios.

DEGREE OF REPRODUCIBILITY IN PERMEABILITY TO RINGER-LOCKE SOLUTION OF TWO NORMAL LIVING, CONTIGUOUS INTESTINE PIECES VARIABILITY IN THEIR PERMEABILITY WITH TIME OF INCUBATION.

Normal guinea-pigs, weighing 300 g. to 350 g., were starved 24 hours prior to experiment and anaesthetized with intramuscular nembutal at 50 mg./kg. body-weight. After laparectomy, pieces of intestines, 2 inches long were cut out one immediately at the ileocæcal junction, and another contiguous to it towards the jejunum. In a few cases a third piece was also cut out about 6 inches away towards the jejunum and the pieces set up for permeability studies according to Burrows *et al.* (1944). After incubation with Ringer-Locke for 2 hours, during which period the membranes allowed to get stabilized, readings were taken at the beginning and end of 2 two-hourly periods of the position of the meniscus of the Ringer-Locke in the pipette, following its excursions for two minutes before the time and two minutes afterwards. The average of all the readings was taken as the position of the meniscus at the time noted. At the beginning of the first period the pipette was filled with Ringer-Locke till the zero level.

Sera were collected from the animals after cardiac withdrawal of blood and proved to be free from agglutinability against *V. cholerae*.

It will be observed by comparing the values of A_1 and A_2 in either of the two flow periods that for membranes from the same intestine, absolute identity of flow is very impracticable. Since the flow of fluids through membranes is governed by the equation of Poiseuille:—

$$V = \frac{p \pi r^4 t n}{8 L \eta}$$

Date	Volume of Ringer-Locke flown in 1st period from 0.0 c.c. level (2 hours)			Volume of Ringer-Locke flown in 2nd period from 0.0. c.c. level (2 hours)			Percentage diminution in flow of 2nd period w.r.t. flow of 1st period		
	A ₁ (piece near the junction) c.c.	A ₂ (piece next to A ₁) c.c.	A ₃ piece 6" away from A ₁ c.c.	A ₁ c.c.	A ₂ c.c.	A ₃ c.c.	A ₁	A ₂	A ₃
18-10-56	0.91	0.70		0.70	0.47		22	31	
22-10-56	0.67	0.53		0.50	0.28		25	47	
23-10-56	0.74	found leaking		0.39	—		47	—	
24-10-56	0.86	0.99		0.49	0.63		43	36	
25-10-56	1.25	0.85		1.04	0.60		17	29	
26-10-56	0.90	0.51		0.66	0.36		27	29	
27-10-56	found leaking			—	—		—	—	
29-10-56	0.87	0.63		0.56	0.48		36	24	
30-10-56	0.61	0.49		0.30	0.35		50	29	
8-11-56	0.80	0.92	1.35	0.50	0.63		37	32	17
9-11-56	1.61	1.22	2.46	0.58	0.66		50	49	55
10-11-56	0.78	0.49	—	0.66	0.39		15	20	—
12-11-56	0.82	0.63	0.40	0.49	0.39		40	48	10
13-11-56	0.46	0.46	0.55	0.29	0.31		37	33	—

where 'V' is the volume of liquid of viscosity η flowing through 'n' pores of average radius 'r' and length 'L' under a pressure head of 'p' in time 't'.

It can be inferred that under the above experimental conditions, the variations in flow can be attributed to one or both of two entities (a) variation in total number of pores, (b) variation in the length of pores or, in other words, the thickness of the membrane. Under physiological conditions, both these factors can vary. That membranes which are further away from the i.c. valve and nearer the jejunum, decrease in flow rate was found in some cases by comparing a few pieces of the jejunum against similar pieces taken from the ileum (A_3 against A_1). While this cannot yet be generalized, one thing seems to be well established that the same membrane on incubation almost invariably decreases in flow rate and the percentage decrease is as shown in the appropriate columns above.

Burrows (1944) has not recorded the variations in these properties for normal membranes. Since we are engaged in a critical study of the M.P.E. of a large number of *V. cholerae* strains, these basic studies have been undertaken. It is proposed to test the M.P.E. on the same membrane piece after taking its Ringer-Locke flow-rate in the beginning and later with Ringer-Locke containing vibrios, the vibrios to be added to the outside of membrane as permitted by sentence No. 2, paragraph No. 4, page 312 of the *Proceedings of the Society for Experimental Biology & Medicine*, **57**, December, 1944, under the authorship of Burrows.

In looking for any permeability differences, the correction to be made for the old age of the membrane has been indicated by the values noted in the studies.

5. Inquiry on bacteriological investigation of cases of cholera with a view to find out the endemicity of cholera in Calcutta under Dr. S. N. De, Dr. K.C. Basu Mallik and Dr. Mondal at the Medical College & Nilratan Sircar Medical College, Calcutta.

A limited number of unselected cases admitted with diarrhoea and/or vomiting into the cholera ward of Nilratan Sircar Medical College Hospitals were investigated. Fresh catheter specimen of stool or rectal swab, when the former could not be collected, was cultured directly on MacConkey's plates. Previous enrichment on Willson-Blair's modified medium did not prove better. From about 17 per cent (76 out of 453) of the cases *V. cholerae* could be recovered from the stool. Most of the strains belonged to the Ogawa type, and only three were Inaba. The materials from the rest of the cases yielded either pure *B. coli* or *B. coli* mixed with *Proteus morgan*, *B. faecalis alkaligenis*, a typical *B. coli* and occasionally *N. A. G. vibrios*.

LEPROSY

1. Leprosy inquiry under Dr. N. Mukerjee at the School of Tropical Medicine, Calcutta.

During the year under report investigations were carried out on the following lines :—

(i) The electron and phase microscopic studies have been continued in collaboration with the Biophysics Department of the Institute of Nuclear Physics of the Calcutta University. Investigation on the morphology of *Mycobacterium lepræ* from cases without any anti-leprosy treatment showed that each bacillus possessed a thin enveloping membrane around it evidently the bacillary membrane, various morphological forms during the process of growth and multiplication of the bacilli and the relationship of the bacilli with cutaneous nerve endings. The bacilli were found in endorgans of touch corpuscles, inside the myelin sheath of axons, in Schwann cells, attached to axons inside touch corpuscles and also along cutaneous nerve fibres. The changes in the cell membrane and cytoplasm of *M. lepræ* from cases under sulphone therapy, though not very significant, yet suggest further study in this line.

(ii) The cytochemical study was undertaken to locate and confirm the morphological structures detected by electron and phase microscopy and to find out the chemical constituents of those structures. The investigation showed that (1) the polar bodies of the bacilli containing cytochrome oxidase were the mitochondria, (2) the Feulgen positive central zone was the site for nucleus and contained desoxyribonucleic acid (DNA) and showed positive alkaline phosphatase reaction, and (3) the cytoplasm of the bacillus contained ribonucleic acid. It was also observed that the DNA often concentrated towards either pole of a dividing nucleus simulating a mitotic figure. The alkaline-phosphatase reaction was found to be stronger in a dividing nucleus than in a resting one. The nucleus of bacillus was found to contain acid polysaccharides, whereas the bacillary membrane showed the presence of mucopolysaccharides. The lipids in the bacillus was found both in free and bound forms mostly present in polar bodies and cell wall.

(iii) Immunological study with an antigen prepared from a culture of Kedrowsky's bacillus by pyridine extraction followed by acetone precipitation showed similar early immunological skin reaction as that of Dharmendra's refined lepromin in 280 persons tested with the two antigens. Of the 280 persons, 102 were lepromatous cases of leprosy, 129 tuberculoid, 31 maculo-anæsthetic and 9 borderline cases, and the remaining 9 persons had no sign of leprosy. The negative reaction obtained with new and refined antigen in 100 and 99, respectively, out of 102 lepromatous cases, indicate very strongly a similarity of the new antigen to the specific antigen of *M. lepræ*. The new antigen has, however, an advantage over the refined lepromin. *M. lepræ* is uncultivable and bacilli are very difficult to be separated from leprous tissue. Kedrowsky's bacillus

being easily cultivable, the antigen can, however, be prepared in a pure form and in any required quantity. The investigation on the exact chemical nature of the antigen is in progress.

(iv) *Study with radio active DDS tagged with S-35.*—A preliminary study on the uptake of this substance by the leprosy cases was carried out in collaboration with the Biophysics Department of the Institute of Nuclear Physics. A tracer study in 22 cases showed that the concentration of the drug in blood, bone-marrow, healthy skin and nerve was of the same order but the uptake of the drug by the leprosy skin tissue was found to be about 10 times more than the healthy skin. The excretion through kidney accounted for about 78 per cent of the total dose administered. The average rate of daily decrease of the drug from blood was found to be about 62 per cent, whereas excretion through the kidneys was 42 per cent daily. Further investigation with the help of auto-radiography and tracer technique is in progress.

(v) The histochemical studies revealed that the bacilli and their host (macrophages) contain mucopolysaccharides of the same nature, which may probably help to explain at least one of the many probabilities of the intracellular parasitism of leprosy bacilli and their presence mainly in macrophage cells of leprosy lesions. Further investigation on the intracellular lipoids showed that the lipoids in the vacuolated cells were of lipoido-calcium complex form. The alkaline-phosphatase activity of lepromatous tissue showed definite increase in the nuclei of cells, nerve fibres and endothelial lining of blood vessels where bacilli were found in large numbers. The study of histamine content of the tuberculoid and maculo-anæsthetic lesions showed a high concentration of histamine in tuberculoid lesions but in maculo-anæsthetic lesions it was found to be almost at par with that of the healthy skin. The histochemical investigations are in progress to elucidate the nature of host-parasite relationship and enzymic activities responsible for the production of such condition.

(vi) Therapeutic studies with the combined use of INH and DDS in the treatment of leprosy appeared to have produced better results than either of the two alone. Treatment of trophic ulcers with Novolep, a by-product in the manufacture of Novophone (DDS) in 22 cases of leprosy, showed complete healing of ulcers in 15 cases without any bony involvement but significant improvement was not obtained in the remaining 7 cases with underlying necrosed bone.

2. Leprosy inquiry under Dr. V.R. Khanolkar at the Tata Memorial Hospital, Bombay.

Histological study of macules in 30 contacts of leprosy patients was carried out. Out of these, 16 were 'positive contacts', 11 were 'negative contacts' and in the remaining 3 cases no definite conclusion was possible. Histologically, they fall in 3 groups according to (1) no obvious change from the normal, (2) infiltration of histiocytes around blood vessels, and (3) cellular infiltrative exudate around nerve and nerve damage.

From comparative study done so far on the effects of DDS and Neustab show that with DDS there is a progressive improvement both clinically and histologically ; while with Neustab relapses or no marked improvement were noticed either clinically or histologically in the condition of the patients.

Clinical and histological studies on the lepromin reaction done on 53 lepromatous and 34 tuberculoid patients reveal that in early reaction of lepromin in both the types of patients no marked differences were observed except acute inflammatory reaction at the site of injection. In late reaction a definite nodule formation was, however, seen in the tuberculoid cases, whereas in lepromatous cases no nodule was noticed clinically. Histologically, in majority of lepromatous cases no residual histological reaction was seen but in majority of tuberculoid cases formation of focal epithelioid cells reaction with body giant cells and few mononuclear and plasma cells were observed.

3. Leprosy inquiry under Dr. N. Figueredo at the Acworth Leprosy Home, Bombay.

I. RESULTS OF EXAMINATION OF CONTACTS

(a) Examination of 343 family members (contacts) of patients revealed that 20.69 per cent had already contracted the disease, 15.45 per cent were in the infected stage, 9.61 per cent had the earliest lesions and 54.23 per cent were uninfected.

(b) *Lepromin test*.—

- (1) The test was positive in 27.42 per cent and negative in 72.57 per cent of the uninfected contacts.
- (2) The test was positive in 88.68 per cent and negative in 11.32 per cent of the infected contacts.
- (3) The test was positive in all those with early lesions (except one with a primary lesion).

(c) *Development of lesions*.—The results of observation and examination of uninfected and infected contacts and those with the earliest lesions examined in previous years, revealed the following :—

- (1) 24.39 per cent of uninfected contacts got infected.
- (2) 17.08 per cent of uninfected and 37.5 per cent of infected contacts developed primary lesions.
- (3) 14.63 per cent of the uninfected, 4.54 per cent of the infected and 13.33 per cent of those with primary lesions developed anæsthetic or tuberculoid macules.
- (4) 43.9 per cent of the uninfected remained uninfected.
- (5) 36.36 per cent of the infected remained infected.
- (6) 46.66 per cent of those with primary lesions remained unchanged.

(d) Regression of lesions.—

- (1) 18.18 per cent of infected became negative (without treatment) and the lepromin test was still positive.
- (2) The primary lesions in 29.4 per cent vanished (without treatment).

II. RESULTS OF EXAMINATION OF NON-LEPROSY CASES

A few acid-fast bacilli were found on the first examination in 5.28 per cent (11 out of 208) of persons examined on suspicion of leprosy and who were not suffering from the disease. On repeated examination of 36.36 per cent of the cases, none showed acid-fast bacilli.

These results, when compared to those obtained in the case of contacts, reveal a significant variation and provide additional support to the opinion that the acid fast bacilli found in contacts are *M. lepræ*.

III. PROPHYLACTIC TREATMENT AND CONTROL

(1) A group of 74 uninfected contacts were given prophylactic treatment with DDS in varying doses of 5 mg., 10 mg., 25 mg., and 50 mg. (according to age), twice a week, and could be kept under observation for periods ranging from 3 months to 2 years. None has so far got infected or developed lesions.

A control group of 17 could be kept under observation for similar periods. Of these, 6 got infected, 4 developed primary lesions. In 1 out of these 4, the primary lesions vanished later. Four developed lesions with sensory changes and in 3, there was no change.

(2) A group of 43 infected contacts (without lesions) were given DDS treatment in prophylactic doses and could be kept under observation for periods ranging from 3 months to 2 years. Nineteen became negative for bacilli and 24 remained positive.

A control group of 8 infected contacts (without lesions) could be kept under observation for similar periods. Of these, 3 became negative for bacilli, 2 developed primary lesions. In 1 out of these 2, the primary lesions vanished later and in 3 there was no change.

IV. TREATMENT WITH DDS AND I.N.H. COMBINED

543 lepromatous and 61 tuberculoid cases received DDS and INH combined, regularly for a period ranging from 7 months to 1 year.

In the lepromatous group 142 developed lepra reaction and 42 were found to have anæmia during the course of treatment. Forty-eight improved bacteriologically and 6 were rendered negative. There was no change in the remainder.

In the tuberculoid group, 6 developed a reaction and 1 was found to have anæmia during the course of treatment.

One improved bacteriologically. There was no change in the remainder.

The treatment is being continued.

4. Inquiry on the metabolic studies on human leprosy under Dr. Beatriz M. Braganca at the Indian Cancer Research Centre, Bombay.

Studies in progress during the period under review consist of experiments aimed at developing procedures for the separation of human leprosy organisms from the lepromatous nodule in a form which retains the activity of the respiratory system of the organism. For this purpose the differential centrifugation procedures have been employed. The techniques followed are based on those employed in recent years for the separation of various cytoplasmic components. Results obtained from few initial experiments appear to be hopeful, in so far as that the final fractions showed only acid-fast organisms and practically no cellular material when appropriately stained.

5. Leprosy research work under Dr. N. Mukerjee at the School of Tropical Medicine, Calcutta.

Besides the routine clinical and therapeutic work, teaching and publication of the quarterly journal "Leprosy in India", the following research activities were carried out during the year :

Histological studies : The work commenced last year on the Study of the Cutaneous Nerves in Sections from Skin lesions of the two main types of leprosy, was continued. Biopsy material from skin lesions of 132 cases of leprosy (44 tuberculoid and 88 lepromatous), as well as from the normal skin of 14 unaffected individuals were studied. All the biopsies had been done on the forearm. The pathological changes in the cutaneous nerves in such biopsies were studied by staining the nerve either by modified Gass's (1954) method of silver impregnation or Gomori's (1950) method for the presence of acid phosphatase. The position of the lepra bacilli with respect to the nerve-fibre was studied by superstaining the stained section by Ziehl-Neelsen method. Silver staining was done on 51 biopsies and acid phosphatase on 95. Six selected slides from each biopsy material were examined.

Nerves could be detected in 13 of 14 normal, 18 of 44 tuberculoid, and 50 of 88 lepromatous biopsies. It can therefore be seen that the frequency of finding nerve is low in lepromatous and lowest in tuberculoid lesions.

The availability of nerves with respect to the duration of the lesions was compared in 25 tuberculoid and 19 lepromatous sections

stained by the acid-phosphatase method. It was found that in both types of the disease the frequency of finding nerve decreased with the duration of the lesion and this decrease manifested earlier in the tuberculoid lesions.

The availability of nerve in relation to the degree of infiltration in the lesion was compared in 21 tuberculoid and 19 lepromatous sections stained by the acid-phosphatase method. It was found that the frequency of finding nerve in section diminished in both types of leprosy with the degree of infiltration, and this diminution was more pronounced in the tuberculoid lesions.

The above findings confirm the general view that destruction of cutaneous nerve is more common in the tuberculoid lesions, and there seems to be a positive correlation between this destruction and the duration and degree of infiltration of the lesion.

Regarding the pathological changes in the nerves (i) in the tuberculoid lesions, the dense and compact perineural infiltrate appeared to enter into the nerve to become endoneural, causing fragmentation and fraying of the fibres. The axons were stained uniformly throughout its whole length, either of dark or lighter colour. It is probable that as a result of such fragmentation, there is Wallerian type of degeneration in the distal portion of the fibre, (ii) in the lepromatous lesions, on the other hand, there was only a loose perineural cuffing of infiltrate without any appreciable endoneural infiltration. The nerve-fibres were not fragmented, were wavy in outline, parallel to and equidistant from one another, as in normal skin. But the axons were not uniformly stained throughout its whole length, some portion taking up darker stain, whereas the adjoining portion may be lightly stained. It is probable that parenchymatous type of degeneration occurs in the fibres as a result of slow compression by the perineural cuffing of infiltrate.

For studying the relation of the bacilli to the nerve-fibres, 4 tuberculoid and 10 lepromatous sections stained by acid-phosphatase method and showing nerve, were superstained by Ziehl-Neelsen method. In both types of lesions, the bacilli were found to lie in between the nerve-fibres along the inter-fibre spaces, the number of bacilli being few in the case of tuberculoid lesions. In the lepromatous lesions, where as a rule there are large number of bacilli and some globi, no bacilli could be found within well-stained axons. In case where the fibre was very lightly stained, a few bacilli appeared to lie also within the Schwann cell or myelin sheath. It appears that probably the bacilli are unable to penetrate into the various parts of a nerve-fibre with normal metabolic activity.

THERAPEUTIC STUDIES :

Two indigenous medicines were given a limited trial in the treatment of leprosy. (i) "Lepravasin" containing a mixture of sulphides or copper, iron and gold, and (ii) "Leprazyme"—containing a lipolytic enzyme. The therapeutic efficacy of both the drugs has been tested on about a dozen cases of leprosy (comprising of about equal number of lepromatous and non-lepromatous types), the maximum period of

treatment extending upto 5 months. Both the drugs were given daily—lepravasin orally in dose of $1/4$ grain, twice daily, and “lepra-zyme” subcutaneously or intramuscularly in doses of 5 to 15 c.c. No beneficial effect was observed in either case.

Antigene Marianum.—Therapeutic trial of an antigen prepared from a culture of *Mycobacterium marianum* has just been started. 10 cases of leprosy (6 tuberculoid and 4 lepromatous) have been kept under observation. The antigen is given in doses of 0.1 c.c. intramuscularly on the arm at intervals of one month. 3 injections have so far been given to each of 8 patients, and 2 injections in the remaining 2 cases. The period of observation is only 2 months. In every case, local reaction at the site of injection in the form of erythema and induration was found and this reaction was more pronounced with a tendency to ulcerate at each subsequent injection. No focal reaction has been found in any, except in one lepromatous case who developed a general reaction after the second injection. General reaction in the form of fever was found in only two lepromatous cases. Slight improvement in the clinical condition has so far been noticed in all but 2 lepromatous cases—including the one who developed general reaction. The trial is continuing.

MALARIA

1. Insecticide & mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi.

During the period under review the following laboratory and field investigations were carried out :

A. LABORATORY INVESTIGATIONS :

1. Development of resistance in houseflies and mosquitoes to chlorinated hydrocarbon insecticides, in Malaria Institute of India, Delhi.

(a) Experiments were continued on the development of resistant strains of *M.nebulo* and *C.fatigans* to DDT, BHC and DDT and BHC combined by exposing insects of each generation to the deposits of respective insecticides. The work has progressed through 45 generations in case of *M.nebulo* and 37 generations in the case *C.fatigans* so far. Medium lethal concentrations of insecticides were worked out which showed that the selected strains compared to normal strains have developed higher tolerance to the respective insecticides.

(b) DDT, resistant strain of *M.domestica* is also being selected by exposing the insects to DDT at each successive generation. Compared to the normal strain signs of higher tolerance to DDT were first observed in the 5th generation. The resistant strain has so far progressed through 6 generations.

Fluctuations in the level of tolerance to the insecticide, by the selected strains of *M.nebulo* and *C.fatigans* as well as the normal strains have been observed in different generations. These preliminary

observations are being pursued and the cause or causes responsible for the variation are under investigation.

2. *Development of resistance to DDT in C. fatigans, A. fluviatilis and A. stephensi. in Malaria Institute of India (Southern India Branch, Coonoor.)*

(a) *C. fatigans*.—In order to find out whether resistance built up through exposure of adults was also demonstrable in the immature stages, comparative tests were carried out against full grown larvae of resistant and non-resistant strains of *C. fatigans*. There was unmistakable evidence to show that the larvae, too, were resistant to the insecticide. The concentration of DDT which caused cent per cent mortality among larvae of non-resistant strain was completely ineffective against larvae of the resistant strain.

(b) *A. fluviatilis*.—As already reported, resistance in *A. fluviatilis* was very late in appearing and very slow in building up. This strain has passed through 57 generations so far. The amount of resistance developed is of a low order.

(c) *A. stephensi*.—Experiments concerning the speed of development of resistance in *A. stephensi* were started by exposing (i) larvae only, (ii) adults only and (iii) both larvae and adults, assuming that resistance could be developed in the species under laboratory conditions. These studies are well under way.

3. *Efficacy of Diazinon and Malathion residual sprays against flies and mosquitoes.*

Toxicity of Diazinon and Malathion as residual sprays was evaluated at Delhi and Coonoor.

(a) *At Delhi*.—Biological efficacy of Diazinon and Malathion against laboratory bred normal strains of *M. nebulosus* and *C. fatigans* was assessed on glass panels sprayed with these insecticides at the rate of 25 mg./sq. ft. Both Diazinon and Malathion were found to be effective against *M. nebulosus* and *C. fatigans* for 7-9 weeks. Further work on the duration of their residual toxicity on mud surfaces is in progress.

(b) *At Coonoor*.—Tests concerning the toxic action of diazinon and malathion (25 mg./sq. ft.) against resistant and non-resistant strains of *C. fatigans* were carried out under laboratory conditions. Both these insecticides were found to be effective against normal as well as DDT resistant *C. fatigans*.

B. FIELD INVESTIGATIONS :

1. *Studies on the comparative efficacy of different insecticides against mosquitoes :*

(a) *1955 Field trials*.—Field studies on the comparative efficacy of DDT (100 mg./sq. ft.), BHC (20 mg. gamma isomer/sq. ft.) dieldrin (25 mg. and 12.5 mg./sq. ft.) and malathion (25 mg./sq. ft.) residual sprays against mosquitoes were carried out during 1955 in the village of three States of India, viz. Madhya Pradesh, Madhya Bharat and

Vindhya Pradesh. All the four insecticides were tried in the form of water dispersible powders. During the period under review the studies were completed and the data analysed.

In all the States, the areas selected were such where *A. culicifacies* appeared to be the main vector of human malaria. In Vindhya Pradesh *A. fluviatilis* in addition played an important role in the transmission of the disease.

From selected villages entomological and epidemiological indices were collected for the evaluation of results. It was found that dieldrin, DDT and BHC at the dosages applied had about the same period of residual toxicity against mosquitoes of about 8 weeks except for DDT in Vindhya Pradesh which was effective throughout the period of 11 weeks of observation. Malathion at a dosage of 25 mg. per sq. ft. was least effective as a residual spray, the duration of effectiveness lasting for only 3 weeks in Vindhya Pradesh and 6 weeks in Madhya Pradesh. Results in Madhya Bharat with this insecticide were inconclusive because of the natural decline in the density of mosquitoes.

(b) *1956 Field trials.*—Field studies on the comparative efficacy of DDT 50 per cent compared to DDT 75 per cent water dispersible powder dieldrin 75% compared to 50% water dispersible powders with and without resin; BHC 6.5 per cent compared to 50 per cent gamma isomer water dispersible powders and diazinon 40 per cent water dispersible powder were undertaken in a group of villages in Meerut District, (Uttar Pradesh). Nine villages were selected for these trials eight of these were sprayed with insecticides under test and the ninth village was left untreated for purposes of comparison. The dosages employed were DDT 100 mg., dieldrin 25 mg. BHC 20 mg. gamma isomer and diazinon 25 mg. per sq. ft.

From the perusal of the entomological data so far collected preliminary conclusions are as under :

Out of the two DDT formulations DDT 75 per cent water dispersible powder appears to be slightly more effective; of the two BHC formulation 50 per cent gamma isomer gives better results. Among the three dieldrin formulations, dieldrin 75 per cent seems to be better and between the two 50 per cent dieldrin water dispersible powders no definite conclusions could be drawn*. Diazinon though initially very effective does not have prolonged residual effect. Final analysis of the data will be made on the conclusion of the observations which are still in progress.

2. *Preliminary observations on the efficacy of diazinon and malathion to control breeding of culicine mosquitoes.*

Field studies on the effectiveness of diazinon and malathion (both as suspension and emulsion in water) against culicine larvae were

* The village sprayed with 50 per cent dieldrin water dispersible powder with resin was unfortunately sprayed with DDT through oversight by the local squads. The results, therefore, could not be taken into consideration.

undertaken in Shahdra area of Delhi State and Ghaziabad area of Uttar Pradesh during 1956. The experiments were carried out on sullage water collections in the form of small pools. Larva density was recorded in all water collections before and after insecticidal application.

In the case of diazinon suspension and emulsion the effect of the treatment lasted for periods varying from 2 to 21 days in the different breeding places.

Larvicidal effect of malathion suspension and emulsion was found to last for 2 to 19 days in different breeding places depending upon their type.

Out of the three insecticides tested only DDT was not effective even for a single day in some breeding places. Considerable variation was noticed in the duration of larvicidal effectiveness of both diazinon and malathion in different breeding places. It was observed that in the case of these two insecticides the larvicidal effect of the treatment was more persistent in water collections where there was no inflow or outflow of sullage water, where organic pollution of the water was not of a very high order, and where there was little scum formation on the water surface.

3. *Efficacy of diazinon as a residual spray against houseflies.*

For these studies, houses in two slum areas (Qadam Sharif and Basti Bazaria) of Delhi were sprayed with diazinon (25 mg./sq. ft.) and for purposes of comparison with BHC (10 mg. gamma isomer/sq. ft.). Suspension of insecticides in water were used. Fly densities were recorded from the two sprayed areas and for purposes of comparison from a similar untreated area (Ramnagar). The data so obtained indicated that with diazinon application there was considerable reduction in fly population and fly density remained low for about one week after insecticidal application. The results with BHC application were not so encouraging as even on the 1st day after its application, reduction in fly density was less than 50 per cent.

4. *Development of resistance to DDT in C. fatigans larvae in a village near Coonoor (Madras).*

Comparative tests for susceptibility to DDT were carried out with full-grown larvae reared from two wild caught females from a village (near Coonoor, Madras) which had been sprayed with DDT for several years and larvae of the normal strain of *C. fatigans* maintained in the laboratory. *C. fatigans* larvae from the village were found to have acquired resistance to DDT.

28. *Inquiry on the preparation of water dispersible DDT under the Director, National Chemical Laboratory, Poona.*

Aim, object and scope of the work.—Water dispersible dry DDT powder, which are usually prepared by grinding of DDT, inert clay, and wetting and dispersing agents, are largely being used under Malaria

Control Programme. The above method of preparation is multi-stage, laborious and requires many costly equipments. Furthermore, the commercial powders contain high proportion of inert material (25 to 50 per cent) as diluents and the DDT particles in them have the undesirable spherical shape, which reduce the covering power. The object of the present work is to prepare similar, more concentrated, powders by a simple process of spray atomization of molten DDT containing wetting and dispersing agents in a single step. This requires comparatively fewer inexpensive equipments. We are also aiming to have DDT particles with an acicular (needle) shape to give them greater covering power as was obtained in our previously reported pastes.

(a) *Preparation of water-dispersible DDT in the form of oil bound as well as aqueous pastes.*—The following two types have been prepared :

- (i) Oil-bound paste containing 70 to 90 per cent technical DDT, and
- (ii) aqueous pastes containing up to 65 per cent DDT and incorporating indigenous saponin as wetting and dispersing agent. These pastes are inexpensive, easy to prepare and contain DDT as needle-shaped particles of larger surface area than spherical particles, and thus have greater covering power and toxicity. Applications have been filed for patenting these processes.

(b) *Preparation of dry wettable DDT powders by a spray crystallization process.*—A new process for the dry powder preparation by spray crystallization is being developed. In this process molten DDT together with wetting agents and other ingredients are passed through a nozzle under pressure and the product is obtained in one stage. One of the most difficult problem in the process, viz. spontaneous crystallization of the molten DDT, has been practically solved by using a suitable nucleating agent. The effect of a large number of potential nucleating agents on the velocity of crystallization of DDT (both pure pp' and technical) has been studied. Several trial experiments have been carried out and both wettable and non-wettable powders prepared. The average particle sizes in these products are between 20μ and 90μ . The suspensibilities of the wettable powders of the desired order have not yet been attained and further work is in progress.

(c) *Measurements of contact angle and sedimentation (volume and rate).*—The results obtained from such studies are utilized to predetermine whether a wetting agent will be suitable for the preparation of a particular stable suspension. A large number of surface active agents have thus been examined for their suitabilities for DDT, gammexane, aldrin, dieldrin and endrin compositions.

Importance of the work.—A simple but inexpensive method for the preparation of water dispersible DDT is of great importance as wettable powders are used in large quantities and these are at present prepared by laborious and expensive methods. The spray atomization is a new technique which is being developed in this laboratory for the above purpose. This work is of fundamental as well as of applied interest.

3. Inquiry on the factors influencing the development of malaria parasite in mosquito by cytochemical technique under Dr. H.N. Ray at the School of Tropical Medicine, Calcutta.

During the period under review the following lines of work were undertaken :—

I. *Effect of growth factors on the sporogonous cycle of Plasmodium gallinaceum in Aedes ægypti.*—A series of experiments were designed to study the effects of different growth factors, such as di-methionine, paminobenzoic acid (PABA), p-hydroxy-benzoic acid (POB) and adenosine triphosphate (ATP) on the development of *P.gallinaceum* in *Aedes ægypti* kept on basal diet of 4 per cent glucose solution.

Observations made so far indicated that methionine stimulated growth of the parasite in the mosquito as evidenced by (i) early appearance of oocysts and sporozoites, (ii) relative increase in size of the oocyst, and (iii) relative intensification of infection. PABA, POB and ATP did not show any stimulating effect to any marked degree. Cytochemical changes of the parasite and the host tissue are under observation.

II. *Comparative cytochemical studies of P.gallinaceum in A.albopictus.*—With a view to explore the host specificity studies were undertaken on the development of *Plasmodium gallinaceum* in the gut of *Aedes albopictus*. It was found that the sporogonous cycle of the parasite developed fully and completely in a similar manner as that of *A.ægypti*. Cytochemical characters of the different developmental forms of the parasite in *A.albopictus* appeared to be similar to those of the corresponding stages developed in *Aedes ægypti*.

III. *Observations on the (i) female gametocyte, (ii) ookinete, (iii) oocyst, and (iv) sporozoites of Plasmodium gallinaceum.*—Detailed observations were made on the female gametocyte and ookinete of *P.gallinaceum* which had so far received very little attention. In female gametocyte no evidence of 'polar' bodies could be detected. A study of chromosomes showed that like most other members of sporozoa, this parasite was also haploid. 'Boomerang' shaped ookinete measured 11.5 micra to 17.5 micra in length and 1.5 micra to 2.5 micra in breadth with nucleus usually situated in the centre.

In continuation of the studies on the cytochemical make-up of oocyst (previously mentioned in the last report), it was observed that what Corradetti (*Riv. di Parasit.*, 1, 89-97, 1937) considered as infoldings of oocyst wall were actually intracytoplasmic inclusions containing lipids as demonstrated by the histochemical reaction with Sudan black B.

Sporozoites of *P.gallinaceum* when stained with toluidine blue after fixation with alcohol-formalin (9:1) in the majority of cases, showed metachromatic dots situated on either side of central nucleus or more than two dots were arranged in a row on one side of the nucleus.

IV. *Mitochondria in Plasmodium gallinaceum.*—Presence of mitochondria was revealed in the trophic, schizont and gametocytic

stages of *P. gallinaceum*. These structures reduced Janus green B to diethylsaffranine and finally to a leucobase indicating the presence of oxidation-reduction activity or some reducing enzyme or chemical in the mitochondria.

V. *Cytochemical studies of the salivary glands and Malpighian tubules of Aedes aegypti*: *Salivary glands*.—The nucleus and nucleolus of both the types of acini showed the presence of DNA and RNA, respectively. Secretion of the central acinus showed Feulgen-positive material. 'Colloid' type of acini gave a more intense reaction for PAS-positive substance than 'granular' type. Nuclei of both the types of glands showed strongly positive reaction for alkaline phosphatase. Faintly positive reaction was also evidenced in the secretion of the 'colloid' type. The work is in progress to ascertain the significance of the different histochemical make-up of the two types of acini.

Malpighian tubules.—The distribution of the enzyme alkaline phosphatase in the Malpighian tubules paralleled to what is encountered in the neprons of the vertebrate kidney. It may be incidentally mentioned that alkaline phosphatase activity in the Malpighian tubules of *A. aegypti* was reduced considerably after a short exposure to benzene hexachloride vapour.

VI. *Effects of post-blood feed on the sporogonous cycle of Plasmodium gallinaceum in Aedes aegypti*.—As a result of post-blood feed to infected mosquitoes it was seen that mammalian and normal fowl blood had stimulating effect on the growth of oocysts of *P. gallinaceum*. Mosquitoes fed in normal fowl blood had fully formed sporozoites in the haemocoel at the end of 12th hour as compared against 148 and 172 hours in glucose-fed batch.

4. **Inquiry on biochemical investigations in malaria with particular reference to protein metabolism of *P. gallinaceum* in chick malaria under Dr. M. Sirsi at the Indian Institute of Science, Bangalore.**

Object of the inquiry.—In spite of the vast amount of attention paid to elucidate fundamental problems concerning the malarial infection, definite data pertaining to the essential requirements of the parasites for its growth and multiplication in the host and the pathogenesis of its protein manifestations are far from clear. The blood forms of the malarial parasite, though located inside the red blood cells, are not intracellular parasites since they obtain part of their requirements from the surrounding plasma. Since a study of the biochemical changes in the blood and tissues of the infected host might throw light on the nutritional requirements of the malarial parasite, the object of the inquiry has been (a) the determination of the biochemical changes in the host with particular reference to protein metabolism. The alterations in the amino acid make-up (free and combined) of the whole blood, red blood cells, plasma, liver and brain in the normal, prepatent and highly parasited conditions in avian malaria (*P. gallinaceum* in chicks).

(b) Since the amino-acid changes are dependant on the protein constituents which vary in the quantitative composition of their amino acids, the result obtained above merely reflects an overall picture without denoting the need of any specific amino acid. Hence, the specificities of amino acids in altering the growth and multiplication of the malaria parasite has been studied by supplementing the amino acids during the course of malarial infection.

(c) The influence of some of these amino acids and the specificity of the reaction has further been clarified by the use of anti-metabolites.

Changes in the free and combined amino acids in the blood (whole blood, plasma, erythrocytes, liver and brain) of chicks in malaria (P. gallinaceum).

Experimental.—Young chicken, 4 to 6 weeks old, hatched and bred under standard laboratory conditions and belonging to the white leghorn or Rhode Island type were used for investigations and the strain of *P. gallinaceum* was obtained from the southern branch of Malaria Institute of India, Coonoor, and maintained by weekly transfers in the Laboratory.

The experimental birds were fed once in the mornings and blood samples were drawn the next morning before giving any diet, thus allowing a period of about 24 hours, to eliminate the effects due to amino acids of the food during the process of absorption. 2 c.c. of blood was drawn in citrated centrifuge tube (10 mg./c.c. of blood).

The changes in the levels of the various amino acids present in blood, plasma and erythrocytes and tissues were determined by the quantitative paper-chromatographic method using three solvent mixtures for development. Hydrolysis of these samples were carried out with 5 N HCl either by autoclaving or by refluxing after preliminary removal of free amino acids with 60 per cent alcohol. The hydrolysates were freed from HCl, neutralized and their nitrogen contents were determined. These were then subjected to chromatographic analysis and the results were expressed as amino acid percentage of the hydrolysate, as percentage of nitrogen. Free amino acids were separately analysed on equal volume basis.

The results so far got indicate that there is a marked change in the concentration of the amino acids (free and combined) present in the whole blood, plasma, erythrocytes, liver and brain at the pre-patent period and at the peak of parasitæmia.

Free amino acids.—A majority of the free amino acids (leucine, isoleucine, phenylalanine, valine, methionine, tyrosine, alanine, threonine, glutamic acid, glycine, serine, arginine, lysine, and cystine) were found to be increased in whole blood during parasitized condition. In plasma most of these were increased in the incubation period and then decreased during infection. In the case of erythrocytes most of the free amino acids (leucine, isoleucine, phenylalanine, valine, methionine, tyrosine, alanine, threonine, glutamic acid, glycine, serine, arginine, lysine and cystine) decreased in the pre-patent period and then increased in the

infected condition. In the case of liver, majority of the amino acids (leucine, isoleucine, phenylalanine, valine, methionine, tyrosine, alanine, threonine, glutamic acid, glycine, serine, arginine, lysine, and cystine) were decreased during the incubation period and then increased during acute infection. In the brain tissue some free amino acids steadily decreased from beginning to the infected condition (isoleucine, phenylalanine, tyrosine, glutamic acid, glycine, serine, aspartic acid and lysine), while others (leucine, valine, methionine, alanine, threonine, arginine, cystine, peptide near cystine band) increased during infection.

Combined amino acids.—In the whole blood among the combined amino acids, histidine, tyrosine, lysine, cystine, decreased in the infected condition, whereas isoleucine and methionine slightly increased. A majority of the amino acids, such as leucine, isoleucine, phenylalanine, valine, alanine, threonine, glutamic acid, glycine, serine, aspartic acid and arginine, however, decreased during incubation period and then increased in parasitized condition. In the case of plasma, a majority of these decreased in infected condition.

In parasitized condition almost all the amino acids of the erythrocytes showed an increase. This general increase might probably be due to the presence of malarial parasites in a larger number of erythrocytes (60 to 80 per cent) whose body proteins also might constitute the protein of the erythrocytes.

Since a general increase of almost all the amino acids are found in erythrocytes in particular, it is clear that the metabolic pathways and metabolic requirements of the parasite are, to a large extent, similar to that of the host. It has been reported by several investigators that free amino acid pool also affects the formation of enzymes and other related co-factors of metabolic processes. Since during infected condition the erythrocyte is a centre of increased metabolic activity, the increase of the levels of amino acids might probably be accounted for as due to the above enzymatic activities.

In the case of liver and brain, the studies so far carried out revealed that a good number of the amino acids were decreased in the liver and brain in parasitized condition. In liver, phenyl alanine, glutamic acid, glycine, cystine were found to be increased in parasitized condition, whereas in brain isoleucine, tyrosine, glutamic acid, arginine and histidine seemed to behave in the above manner.

The effect of supplementation of individual amino acids on the P. gallinaceum infection in chicks.—The influence on the prepatent period and the extent of parasitæmia on chicks infected with *P. gallinaceum* was studied by administering the respective amino acids either orally or intramuscularly.

The observations suggest that amongst the amino acids, threonine, phenylalanine, leucine, histidine, lysine and methionine stimulate the multiplication of the parasites as noticed by the earlier onset and higher parasitæmia in the blood. Isoleucine also brings about an earlier appearance of the parasite but the parasitic count is low throughout the course of infection. Valine, arginine and tryptophan do not exhibit

any marked effect. Proline, aspartic acid, glycine and sodium glutamate delay the onset of parasitæmia and exert inhibitory effect on the multiplication of the parasite.

Amongst the other amino acids, alanine and hydroxyproline show no effect, while tyrosine, cystine, citrulline and serine stimulate the parasitic growth and multiplication.

These results indicate that threonine, phenylalanine, leucine, histidine, lysine, methionine, tyrosine, cystine, serine and citrilline are probably needed for the metabolism of the *P. gallinaceum* parasite. The inhibitory effects shown by some of the amino acids may be either due to a direct toxic action on the parasites or to a stimulatory effect on the defensive mechanisms of the host.

The effect of some amino acid/antimetabolites on chick malaria (P. gallinaceum.)—Since some of the amino acids were found to exert specific effects by stimulating the growth of the parasites and were considered as probably essential for the development of the parasite, the effect of preventing the utilization of these amino acids by administering suitable antimetabolites was next investigated.

The results indicate that ethionine and methionine sulfoxide do not modify the disease, norleucine, norvaline, amino ethane and amino methane sulfonic acids delay the onset of parasitæmia but do not alter the severity of the infection. Only 6-methyltryptophan causes prolongation of the prepatent period and also exerts a beneficiary influence on the severity of infection.

The actual interpretation of the results as to their effects only on the parasite is rather difficult as these antimetabolites also exert their influence on the cellular mechanisms of the host.

5. Inquiry on the study of the life-history, bionomic, taxonomy and disease transmitting capacity of members of family ceratopogonidæ under Dr. B.C. Basu at the School of Tropical Medicine, Calcutta.

The present study deals mainly with the taxonomy of the Indian representatives of the genus *Culicoides* of family *Ceratopogonidæ*, Order Diptera. The problem has been explored to a good extent and a key for identification of the species is being devised. Of the materials collected in and around Calcutta, two new species—*Culicoides palpifer* and *C. alatus* and four described species,—*C. peregrinus*, *C. similis*, *C. anophelis* and *C. quadrilobatus*, have been recorded. Three more species of the genus *Culicoides* are awaiting identification. *C. clavipalpis* described by Mukerji (1931) have been found to be only a synonym of *C. similis* described first by Carter, Ingram and Macfie (1920) from Africa and subsequently recorded by Smith (1932) from Assam in India. *C. similis* in both the sexes have been recorded by us in and around Calcutta. Two instances where a pre-occupied name has been used to describe species of *Culicoides* have been met with and on comparison of their morphological descriptions necessary adjustments have been suggested.

The determination of range of variation of important morphological characters in *C. alatus* have been attempted and it has been found that,

- (i) Variation in body-length is considerable and a range is to be determined for a species on a fair number of specimens.
- (ii) Colour-pattern of haltere is constant in both the sexes of a species.
- (iii) In the wing, little variation exists in size and characteristic spots vary in a few cases.
- (iv) Regarding colour pattern of dorsum of thorax, a fundamental pattern holds true for a species and variation between two sexes is seen in disposition of a few stripes, while within sex it is in intensity of colour of different stripes.
- (v) Spermathecae shows a remarkable uniformity in number, shape and in perfect preparations, also in size.

Antennae and mouth-parts of 8 different species of the genus have been studied. The last antennal segment in both the sexes and the number and disposition of teeth in mandible and galea of female mouth-parts have been found to provide effective data in differentiation of species. A tentative key to differentiate 8 species of *Culicoides* solely on the last antennal segment has been prepared.

The male genitalia has been studied in 7 species and on comparison of data accumulated thereupon with descriptions given by previous workers on other 7 species, preparation of a key on basis of male genitalia is being attempted.

Besides, studies on some aspects of life-history and bionomics of a few species have been made. Regarding infestations of mosquitoes by *C. anophelis* and of *C. anophelis* and *C. palpifer* by mites some interesting observations have been recorded.

6. Inquiry on studies in enzymes of *pasteurella pestis* under Dr. D.L. Shrivastava at the Central Drug Research Institute, Lucknow.

In addition to the detailed work on (a) deamination of amino acids, and (b) alkaline phosphatase of the virulent and avirulent strains of *Pasteurella pestis*, reported last year, it has been possible to complete the detailed investigations on.

- (1) Oxidative metabolism of the virulent and avirulent strains.
- (2) Transamination reactions in both virulent and avirulent strains.

The salient findings are as under :—

1. *Oxidative metabolism*.—The oxygen consumption using the conventional Warburg technique with 8 strains each of virulent and

avirulent cultures of *P. pestis*, using 5 amino acids and 3 sugars showed that serine, glycine and cysteine were capable of being oxidatively metabolised by this organism. Incidentally these amino acids were the ones to be appreciably deaminated also. The oxidation rate by a majority of cultures was maximum for serine. Threonine, structurally similar to serine, also showed a rapid rate of oxidation.

Among the carbohydrates, glucose and fructose, were in general better metabolised than ribose. A comparison of the oxidative metabolism between the virulent and avirulent strains did not show any general relationship between virulence and rate of oxygen consumption.

The effect of antibiotics and sulpha drugs on the oxidation of serine and glucose by resting cells of *P. pestis* showed a very strong inhibition by achromycin, aureomycin and terramycin, whereas streptomycin and sulpha drugs, which are used in the treatment of plague, exhibited comparatively slight inhibition of the oxidation of serine. In the case of the oxidation of glucose, however, they showed slight activation.

2. *Transamination reactions*.—Since in our earlier studies the resting cells of *P. pestis* could only appreciably deaminate serine, glycine and cysteine and none of these except cysteine is supposed to be essential for the growth of this organism, it seems possible that this pathogen obtains the energy for its growth in an amino-acid medium, not through simple deamination, but through a different pathway. In view of the important role of transamination in protein synthesis and in intermediary amino-acid metabolism, studies were undertaken to detect the different transaminase systems present in this organism. Such an investigation revealed the presence of enzymes catalysing the conversion of α keto-glutarate to glutamic acid with a number of amino donors, the most effective being the L-isomers of phenyl-alanine, alanine, and aspartic acid.

The optimum pH for transamination reactions with phenylalanine, alanine, aspartic acid and leucine was found to be 8.0, though aspartate- α -keto-glutarate system was comparatively less affected by changes in pH.

The present studies also showed that the alanine- α -ketoglutarate transaminase was totally dependant upon the extraneous supply of pyridoxal phosphate. Its activity was completely lost in the absence of this co-enzyme and proportionally increased with the concentration of the pyridoxal phosphate up to 30 μ g. in the reaction mixture.

Inhibition studies on transaminases showed that the alanine α -ketoglutaric acid system was much more susceptible to almost all the inhibitors used. Special mention may be made of the effect of semicarbazide hydrochloride, iodo-acetic acid, isonicotinic-acid hydrazide and hydroxylamine which showed the greatest inhibition in the two systems, namely, the alanine and aspartate- α -ketoglutaric acid system.

Experiments with chloromycetin, streptomycin, aureomycin, neomycin, sulphadiazine and sulphapyridine showed no action on the

transamination reactions of the plague bacilli and the amount of glutamic acid formed with these compounds was always 5 per cent of the value obtained for the control.

A comparison of the transaminase activity using six virulent and six avirulent strains of *P. pestis* using L-alanine and DL-aspartic acid as amino donors showed no general correlation between virulence and transamination, though all the strains were capable of catalysing this reaction significantly.

In addition to the above, work is already in progress on the following enzymes and is hoped to be completed during the current year.

(a) *Dehydrogenase activity in P. pestis*.—Preliminary studies on the dehydrogenation of a few substrates using 1 sugar, 8 intermediary metabolites and 5 amino acids by *P. pestis* cells showed the best dehydrogenase activity with glucose. Among the metabolites lactate, isocitrate and succinate, being next in order, showed more or less the same dehydrogenation. The pH optimum was found to be 7.0 for both virulent and avirulent strains.

Inhibition studies proved achromycin, aureomycin and terramycin to be potent inhibitors for succinate dehydrogenation in *P. pestis*, whereas chloromycetin was moderately effective and dihydrostreptomycin and neomycin were almost without any action.

(b) *Metabolism of purine and pyrimidine compounds*.—The results so far obtained have established a significant difference between the metabolism of *P. pestis* and *Vibrio cholerae*. Adenosine and adenylic acid were best deaminated by the resting cell suspension of *V. cholerae*, whereas in *P. pestis*, cytidine and cytidylic acid serve as the best source of energy.

7. Inquiry on the mechanism of the maintenance of plague infection in rats and fleas during the interepidemic period and its possible role on the secular trend of plague epidemiology under Dr. S.C. Seal at the All-India Institute of Hygiene & Public Health, Calcutta.

THE SCOPE AND PURPOSE OF THE STUDY

One of the important questions which needed an answer in the epidemiology of plague, as in any other endemic diseases, is whether the secular periodicity was always or often the result of importation or a recrudescence of the simmering or dormant subterranean local infection, in the latter case the knowledge about the mechanism of such infection would be of great interest, particularly from the point of view of effective control and preventive measures. The existing hypothesis did not fully explain the interval between the two outbreaks of plague such as noted in Calcutta, where during a recent investigational work, evidence of the existence of chronic infection of plague in the rat population was obtained. As the workers are generally divided in regard to the

persistence of infection in the commensal rats, one group believing in the recrudescence of infection from the chronic or mitigated plague in the commensal or sylvatic rats and the other not subscribing to this view, it was decided to exploit the observations already made in Calcutta for further investigation on the line.

The purpose of this present investigation was, therefore, to find out the mechanism of persistence of infection among the commensal rats and fleas which would lead to the periodic recrudescence of plague infection without importation. Involved in this study are the question of persistence of infection in fleas, existence of chronic plague in domestic and wild rodents, change of virulence of plague organism, immunity in rats in different seasons, transference of infection through eating of carcasses of carrier rats, etc.

METHODS OF STUDY

These had already been described in the previous report. It was decided to carry out series of transmission experiments with susceptible and immunized batches of local rats, while varying them according to species and method of immunization. Two possibilities were kept in view in connection with the chronic foci that develop, *viz.*, (i) bacteræmia as a sort of relapse followed by recovery, and (ii) relapse followed by acute plague and death. The purpose of experiment with immunized rats was to find out whether chronic foci arise by infecting them naturally or artificially and whether some of them could subsequently infect the susceptible rats through fleas.

The sketches of the component parts of the device made for this experiment were given in the last year's report. Arrangement has been made for largescale breeding of pure strain fleas and for the regular supply of locally captured commensal rats.

SUMMARY OF THE FINDINGS SO FAR MADE

Altogether 13 sets of experiments have been conducted some of which are still continuing at the time of reporting. The results of the first seven series reported last year led to the hypothesis that the organism gets deteriorated as the epidemic proceeds and that the infection is maintained for a prolonged period in the infected animal and that it is possible for the fleas to imbibe infection from such an animal and to transmit it to another susceptible host. The eighth experiment which ran for one year and was terminated at the April this year showed interesting results. Following the start in May 1955 there was a gradual prolongation of the incubation period till January 1956, but it gradually dropped to the original figure within the course of two months—February and March and then rose again in April. The surviving rat was sacrificed at the end of April and was found *P.pestis* positive. The 9th to the 13th experiments have been carried out mainly with immunized rats using *Rattus rattus* and *Bandicota bengalensis* (previously called *gunomys varius*) and two kinds of antigens, *viz.*, the Haffkine plague vaccine and the specific soluble plague protein for immunization.

Opportunities were taken in these experiments to study the behaviour of fleas, particularly in the experiments 9 and 13 during the prolonged interval of 62 and 34 days, and evidences have been obtained which indicate the possible bacteræmic condition prevailing in the immunized rats developing chronic foci when exposed to natural infection.

Another collateral observation which has been made in this connection is that rats immunized with specific plague protein showed greater resistance to infection than those immunized with the Haffkine plague vaccine.

8. Inquiry on the control of *W. malayi* filariasis in Travancore-Cochin under the Director, Malaria Institute of India, Delhi.

Object of the inquiry.—To test the efficacy of diethylcarbamazine in the control of *W. malayi* filariasis.

Work carried out.—The Unit continued its work at Shertallai, Travancore-Cochin State. Studies were concluded in the nine groups of persons put on three dose-schedules reported last year. Three other groups of seven persons each were put on a dose-schedule of 5 mg./kg. single daily dose for 4 days of diethylcarbamazine alone, the same with histantin or calcium. Further, 10 asymptomatic persons with *Mf. malayi* were put on a dose-schedule of 6 mg./kg. of hetrazan alone in a single daily dose for three days. In these groups, microfilarial counts were made a day prior to and in as many as possible, on day following completion of therapy, 25 and 110 days thereafter. In addition to studying the reactions due to therapy, oral temperature at about 10 a.m. was recorded during the period of therapy. Routine blood studies (total rbc, wbc and differential counts) were carried out. In addition, fæces examination before therapy and a month thereafter was also carried out.

In the nine groups put on therapy last year and in the groups placed on therapy this year, "clinical enquiries" are being carried out at the monthly intervals. These refer to the disease states prior to and following therapy.

In addition, a group of 17 persons was placed on single weekly doses of 1 mg./kg. of hetrazan alone for 20 weeks.

The possible role of oral calcium therapy or use of antihistaminics to alleviate or eliminate the reactions due to diethylcarbamazine therapy has also been studied.

Results of studies carried out.—The criteria for selection of dose-schedule suitable for mass therapy were (a) the reduction in microfilarial counts as observed over a maximum period of 110 days, (b) the degree of severity of reactions and especially of fever due to therapy, and (c) the practicability of administration.

(a) *Effect on microfilaria.*—A marked drop in the number of microfilaria was noted in all cases in all groups, as noted from the blood examination

followed up to a maximum of 110 days after therapy (Table I):—

Table I

Effect of various dose-schedules of diethylcarbamazine on the microfilarial counts as observed during a following up period of 110 days.

Dose-schedule	Number of persons observed	Percentage reduction of microfilaria	Percentage negative to microfilarial
A. 2 mg./kg. for 5 days	27	93.0	59.1
B. 3 mg./kg. for 5 days	31	97.5	31.9
C. 4 mg./kg. for 5 days	32	97.2	80.0
D. 5 mg./kg. for 4 days	28	95.2	85.7
E. 6 mg./kg. for 3 days	10	96.4	80.0

with di-ethyl-carbamazine by itself or with calcium or with histantin to the maximum theoretically possible number of reactions expressed as a percentage.

With di-ethyl-carbamazine alone (Banocide or hetrazan)	53.65 per cent
With di-ethyl-carbamazine + histantin.	48.5 per cent
With di-ethyl-carbamazine + calcium	64.04 per cent

Banocide-Ristantin had the least percentage of reactions as compared to di-ethyl-carbamazine (banocide or hetrazan) alone, but the difference is small. Thus, it may be inferred that the drugs had no appreciable effect in the alleviation of reactions. In addition, the desirability of the use of antihistaminics in a mass scale to ambulant persons is an important point to be considered.

Selection of a suitable dose-schedule for mass administration.—An analysis of results set out in Table I will show that dose-schedules C and D recommend themselves for mass therapy. In view of the fact that dose-schedule C however, gives almost as good results when given by itself, it is recommended for mass therapy against *W. malayi*.

Efficacy of single weekly doses of therapy.—A group of 17 persons with *Mf. malayi* were placed on 1 mg./kg. single weekly doses for 20 weeks. The reactions, rise and duration of temperature have been noted during this period and blood surveys carried out in the 5th and 8th weeks of therapy.

An analysis of results showed that the febrile and other reactions were noted after the first and second weekly doses only. In the first

week, 12 of 17 persons had an average rise of 1.38°F on the day after therapy and of 0.34°F on the subsequent day. The corresponding figures in the second week of therapy were 5 of 17 and 0.34°F on day after therapy only. The reaction index was 45.6 in the first and 18.4 in the second week. The percentage reduction in microfilarial count after five single weekly doses was 93.41 and percentage of persons negative was 60.0. The corresponding figures after 8 single weekly doses were 96.4 and 73.3. Due to the shorter period of fever and likely persistence of effect, more studies are proposed in view of the possible use of di-ethyl-carbamazine in such a dose-schedule in tea garden or other controlled labour groups.

9. Inquiry on the epidemiology of filariasis in West Bengal under Dr. P. Sen, Entomologist, Directorate of Health Services, West Bengal, Calcutta.

Check surveys of filarial incidence, microfilarial rate and endemicity position, as well as the vectors responsible in the representative areas of the districts of Birbhum, Bankura and Midnapore have been completed and the investigations of other districts of West Bengal are being pursued.

The actual disease rates of persons showing elephantoid conditions in parts of their body in the localities so far surveyed varied from 2 to 28 per cent depending on the differing epidemiological factors, the average for the areas surveyed within the districts being 14.5 in Birbhum, 19.2 in Bankura and 8.2 in Midnapore. Scrotal involvement was the common occurrence in Birbhum and Bankura, but elephantiasis of the limbs, particularly legs, was the prevailing type in Midnapore.

The microfilarial rate in the peripheral blood varied from 9 to 16 per cent, the highest rate was noticed in the district of Bankura, while in Birbhum and Midnapore this was 12 and 9.8 per cent, respectively. *Wuchereria bancrofti* was the prevailing form in the districts of Birbhum and Bankura, while *W. malayi* predominated in Midnapore.

The mosquito infection rates also varied, to a large extent, depending on season and prevalence of the species concerned in the transmission. *Culex fatigans*, *Armigeres obturbans*, *Mansonioides annulifera* among culicines and *Anopheles hyrcanus nigerrimus*, *A. barbirostris*, *A. aconitus*, *A. pallidus*, *A. ramsayi*, *A. vagus* and *A. subpictus* among anophelines were dissected to find out the role they play in the transmission of the disease. Of these only *Culex fatigans* proved a vector in all the districts wherever bancroftian filariasis prevailed, but in the region of Midnapore (Contai sub-division) where *W. malayi* prevailed, the chief vector appeared to be *A. hyrcanus nigerrimus*. The infection rate in *Culex fatigans* both in Birbhum and Bankura was about 8 per cent. In Midnapore, the overall rate was 3.6 in *A. hyrcanus* and 0.1 in *Culex fatigans*. Infective forms of the filariae were present in both the mosquitos.

Although a large number of *Mansonioides* was dissected, this was not detected to be infective. A short note reporting the findings is awaiting publication.

The work is likely to throw many an interesting result when the survey of other districts is finalized. A filarial map of West Bengal is under preparation.

TUBERCULOSIS

1. Tuberculosis survey under Dr. P.V. Benjamin, Adviser in Tuberculosis, Directorate-General of Health Services, New Delhi.

The Tuberculosis Sub-Committee of the Indian Council of Medical Research was for some time discussing the question of carrying out a scientific survey to find out the incidence of tuberculosis in the country. The Government of India was given a scheme which was to cost approximately Rs. 6 lakhs. In the beginning of 1955, the Government of India sanctioned this scheme and a special grant of Rs. 4.5 lakhs was placed at the disposal of the Indian Council of Medical Research for this purpose.

2. The scheme was examined in detail by the Tuberculosis Sub-Committee of the Indian Council of Medical Research at its meeting held in March, 1955. The Broad outlines of the project were drawn up. Considering the limitations of carrying out a countrywide survey, with the limited resources available, it was decided to confine the survey to find out the tuberculosis morbidity rate by X-raying the population (excluding children below 5 years of age) in the groups selected by the Statisticians on a sampling basis and examining bacteriologically for tubercle bacilli those having significant abnormal shadows in the X-ray. It was also decided that the coverage of the eligible population in the selected groups should be at least 90-95 per cent. For this purpose, it was decided to utilize the available six mobile X-ray Units attached to the tuberculosis institutions at Delhi, Patna, Calcutta, Arogyavaram, Hyderabad and Trivandrum.

3. The area and population of the regions or zones assigned to these units are indicated in Appendix A. The sample selected for the Survey comprised groups from cities, small towns and villages in each zone.

4. Before starting the survey in all the Centres a pilot study was undertaken by the Delhi, Calcutta and Arogyavaram Units to determine the accessibility of villages and blocks and the response of the population of the groups selected. The results of these studies were considered by the Sub-Committee at its April, 1955, meeting. These studies showed that a coverage of 90-95 per cent. was feasible but a good deal of effort and persuasion on the part of the field staff was necessary to achieve that target. In the light of these experiences, a

Manual of Instructions was drawn up for guidance of the field and laboratory staff, and a standard household schedule and X-ray card were prepared. The Manual contained directions regarding the enumeration of the population, reading of X-ray films and the technique of bacteriological examinations.

5. The Delhi and Madanapalle Units had previous experience of field work of this nature and their units started the survey earlier than the other units which did not have such experience and hence had to overcome many difficulties in the initial stages.

6. The progress of the Survey was reviewed from time to time by the Tuberculosis Sub-Committee. The Sub-Committee met twice for this purpose after the last Annual Meeting held in December, 1955.

7. Though the primary object of the survey was to secure reliable data on the incidence of tuberculosis in the various parts of India, it was thought useful to incorporate the results of this survey in a paper on the subjects of 'The incidence of tuberculosis in economically under-developed countries and the methods for evaluating it' which Dr. P.V. Benjamin has to present at the International Tuberculosis Conference held in January, 1957. As this paper had to be ready by the beginning of September, 1956, it was necessary to prepare a preliminary report, based on the survey that could be finished by June, 1956.

8. The work completed by 30th June is indicated in Appendices B and C. The report which has been prepared by Dr. Benjamin and Dr. C. Chandrasekaran as per directions given by the Sub-Committee at its meeting in July, 1956, was furnished to the Indian Council of Medical Research to be presented at the November, 1956 meeting of the Council.

9. The findings of the present report are only tentative. These, however, show that the tuberculosis morbidity in the areas surveyed varies from 7 to 30 for 1,000 population; there is no marked difference as regards the prevalence of the disease in relation to the size of the population or, in relation to villages, small towns and cities; the morbidity rates are lower for females than for males, and that it shows a continuous increase with age. The prevalence in the age group 35 years and over was considerably higher than in the age group 5 to 34 years. The bacillary rate for 1,000 population has shown a variation from one to eleven in the different areas and zones.

10. The data used in the interim report suffer from many limitations which make comparisons between zones extremely difficult. Some of these arise from the fact that the survey is still in progress and that in most of the zones the investigations are far from complete; others are inherent in the methods used for the survey and reflect the technical deficiencies associated with mass diagnostic procedure, e.g. the degree of accessibility of villages or blocks; variations in the reading of X-ray films, techniques used for bacteriological examination and the coverage of the population.

11. The field Survey is now planned to be completed by 31st March, 1957, and the final report of the Survey before the Indian Council of Medical Research Annual Meeting of 1957.

APPENDIX A

Zones selected for survey

Name of zone	Areas included	1951 Census Population
Calcutta	Districts of West Bengal south of the River Ganges and the districts of Mayurbhanj, Balasore and Keonjhar of Orissa State.	23,844,000
Delhi	Punjab Pepsu and Delhi and districts of Saharanpur, Meerut, Bulandshahr, Aligarh and Mathura of Uttar Pradesh	26,691,000
Hyderabad	Hyderabad State, and districts of Sholapore, Bijapore, Belgaum, Dharwar and Kanara of Bombay State.	25,377,000
Madanapalle	Districts of Andhra State south of River Krishna, Mysore State and North Arcot district of Madras State.	23,128,000
Madras	Madras State, excluding the districts of North Arcot South Kanara, Malabar, Nilgiris and Tinnevely.	23,610,000
Patna	Districts of Bihar State south of the River Ganges.	21,554,000 (approximate)
Trivandrum	Travancore-Cochin, Coorg, and districts of South Kanara, Malabar, Nilgiris, Tinnevely of Madras State.	18,775,000

APPENDIX B

Percentage of Population X-rayed

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Name of Zone	Nature of area	No. of units covered	Population in units covered	Children below 5 years	Population eligible for X-ray	Population X-rayed	Percentage X-rayed of eligible
Calcutta	Calcutta City	26 blocks from Calcutta City	14,143	1,146	12,997	11,649	89.6
Delhi	Delhi City	12 blocks from Delhi City	10,984	1,610	9,374	8,460	90.2
	Towns	Selected blocks from 6 towns	13,158	1,940	11,218	10,425	92.9
Hyderabad	Villages	30 villages	30,901	5,240	25,661	24,290	94.7
	Hyderabad City	21 blocks from Hyderabad City	17,741	2,436	15,305	13,848	90.5
Madanapalle	Towns	Selected blocks from 5 towns	11,966	1,751	10,215	9,200	90.6
Patna	Villages	31 villages	24,233	3,504	21,729	19,630	90.3
	Towns	Selected blocks from 5 towns	11,589	880	10,709	8,401	78.4
Trivandrum	Towns	Selected blocks from 2 towns	3,321	485	2,836	1,710	60.3
	Villages	10 villages	26,597	4,135	22,462	18,295	81.4

APPENDIX C

Percentage covered by Bacteriology

Zone	Nature of Area	No. for which bacteriology wanted	No. examined bacteriologically	Percentage covered by bacteriology
Calcutta	Calcutta City	448	211**	47.1
Delhi	Delhi City	419	400	95.5
	Towns	538	510	94.8
	Villages	1302	1233	94.7
Hyderabad	Hyderabad City	370	351	94.9
Madanapalle	Towns	526	493	93.7
	Villages	699	617	88.3
Patna	Towns	317	188**	59.3
Trivandrum	Towns	193	124	64.2
	Villages	666	503	75.5

**Only the cases for which the results of bacteriological examinations were available at the time of the preparation of this report are indicated here.

2. Scheme of tuberculin retesting of mass BCG campaign vaccinated persons in India under Dr. P.V. Benjamin, Adviser in Tuberculosis, Directorate-General of Health Services, New Delhi.

A scheme for re-testing of individuals vaccinated in the Mass B.C.G. Campaign in India was started by the Indian Council of Medical Research in July, 1955, as a continuation of a similar work done by the WHO/TRO team in India during 1954-55.

The team re-tested about 8,500 persons mostly of the age group 5 to 18 years in different parts of the country. Data for 6,835 individuals comprising of 74 groups have been analysed. The following observations are made :—

1. Overall mean induration for all groups has been found with passage time (upto 20 months). It has shown a tendency to increase

thereafter apparently due to the superimposed natural infection. In this higher indurations seem to have been affected more than lower indurations. Non-specific tuberculin sensitivity may be one of the factors responsible for these variations. As these results are based on different batches of vaccine a follow-up study to assess the influence of time on the post vaccination allergy in relation to batches of vaccine has been suggested.

2. Personal factor may also have resulted in variation in production of allergy. A special study has been undertaken in this respect. A report on this will be available only later. However, a preliminary examination of the available data has shown that the mass campaign teams have a tendency to group reactions below 4 mm. as no-reaction. Some of the technicians have also been found to be inconsistent in their reading.

3. A positive correlation has been found between vaccination lesion and the size of induration.

4. Development of keloids has been noted at the site of vaccination; the average size of those keloids was 7 mm.

5. Tuberculin reactions noted on re-testing have been classified according to hardness. Soft reactions predominated.

6. Very few complications followed re-testings.

7. A study of pre-vaccination allergy is in progress. This will also give information on the pattern of non-specific sensitivity to Tuberculin (if any) in different areas.

3. The role of tuberculosis in the pathogenesis of pelvic inflammatory disease under Dr. P.K. Malkani at the Lady Hardinge Medical College and Hospital, New Delhi.

Object of the work.—To make a detailed study of cases of pelvic inflammation and to determine the incidence of pelvic inflammatory lesions of tuberculous origin. This is important from the point of view of treatment. The treatment for lesions due to pyogenic organisms is likely to activate old healed tuberculous foci in the pelvis. Cases diagnosed as pelvic tuberculosis are given anti-tuberculous treatment.

Conclusions.—393 patients of pelvic inflammatory disease were studied.

62 were diagnosed as suffering from tuberculosis of the pelvis.

The incidence of pelvic inflammatory disease of tuberculous origin is 15.77 per cent.

This does not include cases in which the infection from the fallopian tubes has not spread to the endometrium.

Synopsis of the work done during 15 months from 1st July 1955 to 30th September 1956.

The material and methods of study.—The cases of pelvic inflammation are selected from the Gynaecological Out-patient's department. They

are divided into 2 major groups :—

- I Cases of erosion of the Cervix and Chronic Cervicitis.
- II Cases of adnexal inflammation.

Investigations are done on the following lines :

- A. Histopathology.
- B. Bacteriological investigations.

Results of study done so far

	No. of patients	No. of specimens including repeat examination	No. of patients showing evidence of tuberculosis	No of specimens positive for evidence of tuberculosis
1. Patients studied	393		62	
2. Histopathology of endometrial biopsy	393	573	61	148
3. Histopathology of cervix biopsy	84	98	3	3
4. Histopathology of endocervix biopsy	96	109	4	4
5. Ziehl-Neelson stain of endometrial tissue		200	nil	nil
6. Ziehl-Neelson stain of cervix and endocervix biopsy		31	nil	nil
7. Ziehl-Neelson stain of endometrial aspiration smear	324	568	1	1
8. Ziehl-Neelson stain of cervix discharge	326	596	nil	nil
9. Culture of endometrial tissue	41	42	9	9
10. Culture of menstrual discharge	6	6	nil	nil

Adnexal inflammation cases are grouped as follows :—

		No. of cases	No. showing evidence of tuberculosis
1	Unilateral thickening	141	21
2	Bilateral thickening	25	2
3	Unilateral tubo-ovarian mass	145	23
4	Bilateral tubo-ovarian masses	26	12

Culture of endometrial tissue for growth of mycobacterium tuberculosis.—Culture of endometrial tissue of patients previously diagnosed as endometrial tuberculosis based on the histological examination, are sent for culture for growth of Mycobacterium tuberculosis. The culture medium used by the Bacteriologist of New Delhi Tuberculosis Clinic is the American Treadue Society's 'Potato-Glycerine-Egg Medium'. The Bacteriologist of the Vallabhai Patel Chest Institute uses Lowenstein-Jensen medium.

The endometrial tissue of one patient was sent for culture to New Delhi Tuberculosis Clinic on 25th January 1956—the culture report was negative. On 27th February, 1956 a specimen of endometrial tissue of the same patient was sent for culture to the Patel Chest Institute (this specimen was collected while patient was being treated with INH and PAS. She had taken the drugs for 2 weeks). Culture showed growth of mycobacterium tuberculosis.

Treatment.—The results of treatment are assessed by repeated histological examination of endometrial biopsies during treatment and at the end of therapy. The endometrial biopsy is repeated every 4 weeks during the course of treatment. At the end of the treatment the lesion is declared healed if 3 consecutive biopsies done at monthly intervals after cessation of therapy, do not show any histological evidence of tuberculosis. Follow-up biopsies are repeated at interval of 6 months.

Majority of the patients are advised to take INH 40 mg. t.d.s. and PAS 12 g. daily for 12 weeks.

52 patients were advised treatment but only 22 attended the clinic regularly. 12 patients were declared cured. 10 are still under treatment.

The endometrial lesion heals much quicker than the lesion in the cervix. Whereas lesions in the endometrium require about 12 weeks of treatment, the lesions of the cervix do not heal till about 20 weeks of continuous treatment with anti-tuberculous drugs.

4. Inquiry into the virulence to guinea-pigs and mice of tubercle bacilli isolated from tuberculous patients prior to, during and after treatment with streptomycin PAS and isoniazid under Dr. J. Frimodt-Moller at the Union Mission Tuberculosis Sanatorium, Arogyavaram. P. O. Chittor District.

In October 1955 a brief interim report was given at the time of the Indian Council of Medical Research Annual Meeting. The method employed at the present investigation and the results of the first 38 strains of tubercle bacilli were given.

During the last year and up to November 1, 1956, another 82 strains have been tested for virulence on guinea-pigs. The methods used have been the same as previously described. Incorporating the first 38 results with the last 82 tests, the overall results are now as follows :—

TABLE

Results of virulence tests in guinea-pigs

Type of strain	Highly virulent	Virulent	Mode- rately virulent	Attenu- ated	Aviru- lent	Total
Control (H37)	...	1	...	...	...	1
Primary untreated	7	5	10	12	...	34
Treated, sensitive	5	12	10	1	1	29
Streptomycin resistant	2	2	2	7	1	14
Isoniazid resistant	1	3	4	11	6	25
Streptomycin and isoniazid resistant	3	...	3	8	3	17
Totals ...	18	23	29	39	11	120

Fifteen strains have been inoculated into guinea-pigs but the experiments are not yet completed.

Of the strains already completed two were repeated with higher doses, another seven attenuated strains have been tested again after guinea-pig passage on a second series of animals, and two of these have been passed through a third set of guinea-pigs in order to see if the virulence should have increased by the animal passage; this was not the case, the strains remained attenuated. These cultures were tested for drug resistance between guinea-pig passages without any significant change in resistance being observed.

An experiment into the effect of using large adult animals against small, young ones was carried out in order to see how much the age of the animals would affect the virulence tests. For strains, one virulent, one attenuated, one highly virulent and the control strain, H37rv, were inoculated into eight guinea-pigs each; four were young and four were adults. Each set of four were divided into two parts which received 1/1000th and 1/1 millionth milligram culture, respectively. Half the animals, one from each part, were sacrificed at 4 weeks and the remainder after 8 weeks. The results were quite clearcut; the young animals showed more disease than the adults after 4 as well as after 8 weeks, and this principle applied to each of the four strains. This shows that it is important to note the size of the animal and the length of the experiment when assessing the degree of virulence of basis of this type of animal tests.

In order to avoid any effect of different breeds all the animals are bred in the Sanatorium by mixed breeding. If new stocks were bought, the animals were chosen at random in order to confound possible differences in susceptibility.

As several of fresh strains from untreated patients have shown unusual low levels of virulence, the question arises if the guinea-pigs used could have had a special high resistance to infection with tubercle bacilli. In order to determine if the Indian guinea-pigs should possess specially high degree of resistance as compared with European animals, 12 guinea-pigs have been imported from U.K. by courtesy of Dr. Lane-Petter of the Laboratory Animals Bureau, M. R. C. Laboratories, Holly Hill, Hampstead, London. These animals were derived from a strain which is particularly susceptible to tuberculosis. When a sufficient number of animals have been bred, they will be compared with our Indian stocks.

A large number of strains have been tested for catalase activity by the method of Middlebrook.

As for virulence tests on mice, only 8 strains have been tested so far. The first four produced no tuberculous lesions in the mice, although from a few animals tubercle bacilli were recovered. A new stock of mice has been procured from the Indian Science Institute, Bangalore, in the hope of getting a specially susceptible breed of mice.

5. Inquiry on the incidence of human and bovine type of infection in extrapulmonary tuberculosis with particular reference to abdominal tuberculosis with or without associated pulmonary tuberculosis under Dr. N.L. Chitkara and Dr. M.L. Sur at the Medical College, Amritsar.

SYNOPSIS OF THE WORK

This report deals with 43 cases investigated from 1.10.55. to 30.9.56. and 7 cultures pending (from cases who were taken up before 30.9.55.) Cases have been classified according to their clinical diagnosis as follows :—

Intestinal tuberculosis alone	...	16
Intestinal tuberculosis with tubercular lymphadenitis	...	1
Ascites alone	...	1
Ascites with lymphadenitis	...	2
Clandular	...	17
Gold abscess	...	2
Polyserositis	...	1
Pleural effusion	...	1
Tubercular meningitis	...	2

Their ages varied between 5 and 37 years ; duration of symptoms varied from 20 days to 15 years ; 32 of them were males and 11 were females.

In addition to routine hæmoglobin, total and differential leucocyte count, E.S.R., stools and urine, following examinations have been done :—

1. Mantoux test with old tuberculin in the strength of 1 : 10,000 was done in all and was positive in every case.

2. Gastric lavage and/or sputa from all cases were cultured as well as examined by smear and concentration. In 1 case A.F.B. were seen by concentration and smear and in another on culture.

3. X-ray chest was done in 42 cases ; 25 showed no abnormality. 6 showed enlarged mediastinal or root glands. 7 showed parenchymatous lung lesions. 3 showed pleural effusion and 1 showed old healed pleurisy and calcified lymph nodes. Seven of these 17 cases with abnormality in the chest skiagram were of abdominal tuberculosis.

4. Barium-meal study of G.I. tract was done in 17 cases of intestinal tuberculosis ; 12 showed filling defect in caecum and parts of colon. 2 showed small gut obstruction. 1 showed segmentation of meals and 2 showed no abnormality.

5. Barium-enema study of colon was done in 12 cases ; 9 showed filling defect and 3 showed no abnormality. These 3 cases included 1, who did not show any abnormality on barium-meal study.

6. Screening chest of relatives of 34 cases was done. Father of 1 patient showed bilateral apical disease in the lungs, while 2 of the cases

of this series were related to each other and both suffered from tubercular lymphadenitis.

7. Endometrial biopsy was done in 5 cases ; histopathological examination showed tubercular endometritis in 1 case.

8. Stool culture for A.F.B. was done in 3 cases with no growth in any of these. This examination was not done after the first month or so of the period to which this report pertains.

9. Histopathological examination of biopsy material consisting of mesenteric, cervical or axillary glands or intestines was done in 31 cases. Positive evidence of tuberculosis was obtained in 22 cases, hyalanosis after treatment in 1 case, indefinite picture in 1 case and no evidence of tuberculosis in 7 cases.

10. Morbid material was put up for culture in 41 cases. Fourteen gave positive cultures. Another positive growth was obtained from gastric lavage of 1 case.

11. These 15 cultures and additional 7 cultures obtained from cases under investigation before 1.10.55 were typed by animal inoculation and from cultural characters. Fourteen were definitely human type, both from animal inoculation results as well as cultural characters, 6 appeared human from cultural characters and in 2 cases the results are pending.

6. Inquiry on the effect of antitubercular drugs on the synthesis of phthioic acid in *Mycobacterium tuberculosis* under Lt. Col. Dr. S. Chandrasekhar and A.J.H. de'Monte at the V.P. Chest Institute, Delhi.

Several attempts were made to grow *M. tuberculosis* in submerged culture. Submerged cultures were preferred to surface cultures because growth in the former is supposed to simulate growth in the body (low oxygen tension). This did not prove successful. Growth did not exceed 2 g. to 3 g. wet weight/litre. Therefore, in later experiments surface-culture method was adopted. Inoculation method, inoculum, etc., were standardized; 1 mg./c.c. of ground bacilli was the inoculum used. Different modifications of Youman's media were tried. Finally, Youman's medium with 40 g./litre of neutral glycerol and 0.05 g./litre ferric-ammonium citrate was adopted. Up to 32 g. wet weight/litre of tubercle bacilli could be obtained with this. From wet bacterial cells attempts were made to isolate phthioic acid by the following method :—

1. The culture was sterilized by autoclaving at 100° C. for 3 hours.
2. The cells were extracted with ether and alcohol several time. This extracted the lipids.
3. The lipids were saponified by refluxing with alcoholic KOH and the fatty acids separated from carbohydrates and other insoluble material.
4. The mixture of fatty acids was esterified with 5 per cent methanol plus sulphuric acid. This esterified the α -B

unsaturated acids and acids with a large substituent in the α -B position.

5. The fatty acids were extracted with alkali and recovered by precipitation with H_2SO_4 5 per cent and extraction with ether.
6. Acids were obtained by evaporating ether.

From 12.72 g. and 12.06 g. tubercle bacilli wet weight 0.0219g. and 0.0065 g., respectively, of α - β unsaturated acids were obtained. Estimation of C_{27} phthienoic acid was to be made by ultra violet spectroscopy but could not be done because optically inactive (spectroscopically pure) solvents were not available. Efforts are being made to obtain this.

7. Drug Research Scheme under Dr. P.V. Benjamin, Adviser in Tuberculosis, Directorate-General of Health Services, New Delhi.

The Indian Council of Medical Research have in collaboration with the World Health Organisation set up at Madras a Tuberculosis Chemotherapy Project with a view to determine the role of new antibiotics in the treatment of tuberculosis patients while living in their homes even if they live in crowded areas. The Madras Government are also assisting in this research project and are meeting part of its expenditure. The work in the project started in May, 1956.

Till September 1956, 70 patients were admitted for treatment. The contacts of patients admitted are also encouraged to attend the Centre. 203 contacts were X-rayed and given 5 TU Mantoux test. If negative, the 5 TU Mantoux test is repeated and if still negative a 50 TU Mantoux test is performed.

All domiciliary patients are visited at home at least once a week for the first month and, if ill, more frequently. The visiting is done by health visitors under the supervision of the WHO nurse and also the Medical Officer.

The nucleus of the domiciliary service is well established and the families and patients receive frequent and regular instructions on the prevention of spread of the disease, and in sputum disposal. Sputum specimens from the Centre are cultured. The ferric chloride test is routinely used for all the patients receiving 'Pycamisan 10 PH' and has already proved its value in detecting whether patients are failing to take the drug. Patients admitted were given Pycamisan 10 PH. ("PYCAMISAN 10 PH" stands for isoniazid 200 mg. daily in two equal doses, plus PAS (Sodium salt) 10 gm. daily, in two equal doses.) Treatment with Pycamisan 10 PH at home or in sanatorium is now being allocated by randomisation to patients considered to be especially co-operative. Other patients, whose co-operation is suspected, are also being studied at home as a separate group in order to investigate the problems which they present. It is apparent, however, from the ferric chloride test that some patients, particularly when they feel better, are irregular in

taking their medicine. This emphasises the importance of having a tracer substance in isoniazid pills before the administration of isoniazid alone commences, and the possibility of procuring isoniazid in a suitable form from the WHO is being explored.

8. Inquiry to study the cultural characters and pathogenicity of chromogenic acid fast bacilli and saprophytic acid fast bacilli under Dr. Balbir Singh at the Irwin Hospital, New Delhi.

Three strains of saprophytic acid-fast bacilli were isolated from the earth around the laboratory. One strain was isolated by exposing sterile Lowenstein-Jensen slopes in the verandah outside the laboratory. Three saprophytic strains were isolated from the routine cultures of sputum for isolation of *Mycobacterium tuberculosis*.

Sensitivities of these strains and laboratory strains of non-pathogenic acid-fast bacilli (*viz.* *Myco. smegmatis*, *Myco. phlei* and *Myco. stercoris*) against streptomycin, P.A.S. (Para=aminosabicylic acid) and isoniazid (isonicotinic acid hydrazide) were tested.

(2) The concentrates of sputum of 14 patients showing acid-fast bacilli on direct smear examination were inoculated into guinea-pigs.

9. Studies on *Mycobacterium tuberculosis* under Dr. Balbir Singh at the Irwin Hospital New Delhi.

This inquiry was carried out in the Pathology Laboratory, Irwin Hospital, New Delhi, and commenced on the 1st of May, 1956. Standardization of sensitivity tests against the chemotherapeutic agents commonly used in tuberculosis was the subject for investigation during the last 6 months.

A tentative definition of a resistant strain during chemotherapy is a strain that requires for inhibition of its growth higher concentration of a drug than was required by the strains isolated from the same patient before commencement of treatment. Minimal inhibitory concentrations of PAS (para-amino-salicylic acid), streptomycin and/or isoniazid (isonicotinic acid hydrazide) contained in Lowenstein-Jensen slopes (L.J.) that regularly inhibit the growth of all strains isolated from patients who did not have chemotherapy, should provide data to define a resistant strain. Therefore, the scatter of the distributions of sensitivities of pre-treatment strains was determined by tabulating the results of their sensitivity tests.

Methods used for determination of sensitivities.—The methods used in this investigation are those recommended by the Laboratory Subcommittee of the British Medical Research Council for determination of sensitivity of tubercle bacilli during chemotherapy of tuberculosis. Though these methods are open to criticism like those followed by others, the writer has made use of them because of his previous experience with them. Furthermore, it is not within the scope of this investigation to judge the merits of different methods.

Stock solutions.—Stock solutions of Na PAS [sodium salt (dihydrate) of PAS] and isoniazid (Nydrasid, Squibb) were prepared by filtration through chamber-land L3 candle and a stock solution of streptomycin sulphate. (Glaxo) was also prepared with sterile precautions. These were incorporated into the medium before inspissation.

Medium.—Lowenstein-Jensen slopes containing Na PAS in a series of final concentrations from 0.12 $\mu\text{g./c.c.}$, increasing by serial two-fold steps to 32 $\mu\text{g./c.c.}$ were prepared. Similarly, slopes containing streptomycin in a series of final concentrations from 1 $\mu\text{g./c.c.}$, increasing by serial two-fold steps, to 64 $\mu\text{g./c.c.}$, and those containing isoniazid respectively, in final concentrations of 0.1, 0.2, 1.5, and 50 $\mu\text{g./c.c.}$ were prepared. The slopes were inspissated for 1½ hours and were removed from the inspissator on termination of this period.

Inoculum.—The suspension of each strain was prepared by grinding a 3-mm. loopful of the growth in a Griffith tube containing 0.5. of sterile distilled water. The opalescence of the suspension should be equivalent to that of a 10-day old culture in Tween-albumin medium.

Technique.—The suspension was transferred to the L.J. slopes with a 3-mm. loop made of thin platinum wire. A drug-free slope and an agar slope were also inoculated for each strain. A parallel culture of the standard strain, H37RV, was also tested under identical conditions, along with each batch of pre-treatment strains for sensitivity tests. The results were recorded after 28 days incubation at 37°C. The amount of growth on each slope was recorded as confluent (C), innumerable discrete colonies (IM) and actual number of colonies, if their number was less than 100.

Results of the sensitivity tests.—The results were tabulated in 10 tables. Tables I to III showed amount of growth observed in sensitivity tests of pre-treatment strains, respectively, against isoniazid, PAS and streptomycin. Table IV showed degrees of resistance of each strain derived from the ratio between the minimal inhibitory concentrations for the test strain and the standard strain, H37RV. The advantage in determining the resistance ratio is that it is not effected by slight variations in the same technique in different laboratories. Since there are no restrictions on the sale of drugs in this country, it is rather difficult to exclude the possibility of previous treatment with chemotherapeutic drugs by a vaid or a hakim without the knowledge of the patient. It was, therefore, decided to tabulate separately results of sensitivity tests that showed resistant strains according to the definitions adopted by the Laboratory Sub-committee of the British Medical Research Council.

Isoniazid-sensitivity tests.—Eighty-five pre-treatment strains were tested. The growth of a few colonies was observed even on slopes containing 5 $\mu\text{g./c.c.}$ of isoniazid. Therefore, the growth of less than 20 colonies was taken as no growth. The distributions of amount of growth in the tables prepared from the results of these sensitivity tests showed that strains producing more than 20 colonies on slopes containing 1 $\mu\text{g./c.c.}$ or higher concentrations of isoniazid after 28 days incubation

should be considered as resistant and those that produce more than 20 colonies on slopes containing $0.2 \mu\text{g./c.c.}$ of isoniazid should be considered as doubtfully resistant, provided the H37RV shows a lesser amount of growth on $0.2 \mu\text{g./c.c.}$ And sensitive strains are, obviously, those which produce no growth (less than 20 colonies) on $0.2 \mu\text{g./c.c.}$ or higher concentrations of isoniazid after 28 days incubation.

The table prepared from the amount of growth observed in sensitivity tests of a parallel culture of the standard strain, H37RV, done along with 56 batches of pre-treatment strains, showed distributions similar to those seen in the table prepared from the results of pre-treatment strains.

PAS-sensitivity tests.—One hundred and four pre-treatment strains were tested. Complete inhibition of growth did not take place even on slopes containing $32 \mu\text{g./c.c.}$ of Na PAS. The scatter of the distributions of the amount of growth in the tables prepared from the results of the sensitivity tests showed that the strains which can produce more than 20 colonies on slopes containing $8 \mu\text{g./c.c.}$ Na PAS or higher concentrations should be considered as resistant and the growth of more than 20 colonies on slopes containing $4 \mu\text{g./c.c.}$ of Na PAS may be considered as doubtfully resistant, provided the H37RV shows lesser amount of growth on $4 \mu\text{g./c.c.}$ than is shown by the test strain. Sensitive strains are those which produce no growth on slopes containing $4 \mu\text{g./c.c.}$ or higher concentrations of Na PAS after 28 days incubation.

Furthermore, the range of resistant ratios extended from $1/8$ to 4. As none of the strains showed resistance ratio more than 4, a PAS-resistant strain may be defined as one which shows a resistance ratio of 8 or higher than this.

Streptomycin-sensitivity tests.—Ninety pre-treatment strains were tested. The growth of 10 to 19 colonies took place occasionally on slopes containing streptomycin concentration as high as $32 \mu\text{g./c.c.}$ Therefore, as in the cases of PAS and isoniazid, the growth of less than 20 colonies was considered as no growth. The scatter of the distributions of the amount of growth in the tables prepared from the results of sensitivity tests showed that the strains which can produce more than 20 colonies on slopes containing $8 \mu\text{g./c.c.}$ or higher concentrations should be considered as resistant. Sensitive strains are those which do not grow on slopes containing $4 \mu\text{g./c.c.}$ or higher concentrations of streptomycin after 28 days incubation.

The range of resistance ratio extended from $1/8$ to 2 and as none of the strains showed resistance ratio more than 2, a strain showing resistance ratio 4 or higher should be considered as resistant.

The results of the sensitivity tests of pre-treatment strains were consistent with the observations made by workers in other parts of the world that pre-treatment strains are uniformly sensitive to these drugs but for the results of 16 strains that were tabulated separately because they showed strains resistant to streptomycin, PAS and/or isoniazid according to the definitions of resistant strains adopted by the Laboratory

Sub-Committee of the British Medical Research Council. As the analysis of the results of sensitivity tests of pre-treatment strains during the present investigation is suggestive of definitions of resistant strains similar to those adopted by the Laboratory Sub-Committee of the British Medical Research Council, the histories of the patients from whom those strains were isolated, were checked up and those traceable were called for the purpose of this inquiry. The information made available showed that 3 out of these 16 had definite history of previous chemotherapy, one probably had isoniazid and 4 who could be interviewed by the clinician-in-charge of the cases gave no history of chemotherapy before their sputum was obtained for this inquiry. Whether these 4 patients had infection with resistant strains or had chemotherapy without their knowledge is rather difficult to decide. Nor can one draw any conclusion about this from the results of the 8 patients who were not traceable. It was, however, significant that there were only three patients with streptomycin-resistant strains. Two out of these three were not traceable and about the third information is available that he had streptomycin. This is consistent with the fact that both vaidas and hakims do not resort to injection therapy.

Eleven patients showed strains resistant to isoniazid, 6 had strains resistant to PAS (two had both isoniazid and PAS-resistant strains and two others had both streptomycin and isoniazid-resistant strains). Larger number of patients with isoniazid-resistant strains could be due to two reasons. Firstly, because it is a comparatively cheaper drug and is likely to be used more commonly by the quacks. Secondly, because resistance against isoniazid develops much more quickly than against PAS both in *in vitro* experiments and during clinical trials.

10. Inquiry into the susceptibility of guinea-pigs from different parts of India to infection with *Mycobacterium tuberculosis* under Dr. R.K. Goyal at the S.M.S. Medical College, Jaipur.

Object of the work.—The guinea-pig inoculation test is considered to be the most sensitive one for the diagnosis of tuberculosis. Conflicting reports have recently appeared in the literature with special reference to India indicating that the guinea-pigs are fairly resistant to tuberculosis in view of some authors. It is the aim of this inquiry to obtain guinea-pigs from different parts of India to determine if there is really a difference in susceptibility in guinea-pigs obtained from different sources. In case a marked variability is found in the resistance of these animals from different sources, an attempt would be made to determine the factors responsible for this variation in susceptibility.

A suitable strain of tubercle bacilli had first to be selected for the purpose of the experiments. The H 37 strain obtained from one institute did not show a high grade virulence. A number of local strains from untreated cases of tuberculosis was then tried. It was found on a preliminary investigation that growth was noticeable on sub-culture on the Lowenstein-Jensen's medium in 4 to 6 days time in two of the strains. These strains produced definitely rough confluent growths and were of

relatively low virulence. One strain from a case of osseous tuberculosis, which was highly virulent and eugonic was selected for testing the relative susceptibility of guinea-pigs to tuberculosis.

A suitable route of inoculation for the production of lesions had then to be selected. Definite lesions in the omentum, liver, spleen, etc., could be produced by the intraperitoneal injection of even 10 bacilli. The lesions were not marked on intramuscular injection of either organisms of low virulence or when very few bacilli from a virulent strain were injected. From the point of view of comparison, however, of variable susceptibility of guinea-pigs from different parts of India, the intramuscular route gave better results. The macroscopic lesions were confined to the muscle and the regional lymph-nodes in the less susceptible guinea-pigs injected with 10 to 1,000 bacilli, whereas the abdominal viscera were definitely involved when bigger doses were administered, or more susceptible guinea-pigs were used. In the preliminary experiments carried out so far, the results were as follows :

On intraperitoneal injection of the culture of virulent bacilli, there was an earlier mortality and more extensive visceral lesions in the case of guinea-pigs obtained from Kasauli and Bombay as compared with those from Lucknow. The Bombay guinea-pigs were definitely more susceptible than Lucknow guinea-pigs (weight 350 g. to 460 g.), whereas the Bombay guinea-pigs were only 290 g. to 380 g. in weight.

The intramuscular injection of varying doses of tubercle bacilli (ranging from 10 to 10,000 bacilli per c.c.) in Lucknow and Bombay guinea-pigs gave much more clear-cut results. There was a naked eye extension of the lesions into the abdominal viscera in the case of Bombay guinea-pigs, whereas the abdominal viscera remained more or less normal naked eye in the case of Lucknow guinea-pigs injected with 10 to 10,000 bacilli. The intramuscular injection of 10,000 bacilli in the Lucknow guinea-pigs led to the appearance of slight visceral lesions. On histopathological examination, a few foci of reticulo-endothelial hyperplasia could be detected in the liver and spleen of Lucknow guinea-pigs, although these organs were apparently normal to naked eye examination.

In carrying out the Mantoux test in guinea-pigs, injection of a 1/10 dilution of old tuberculin is very satisfactory from the point of view of eliciting the presence of the minimal degrees of allergy. In order to detect the existence of varying degrees of susceptibility to tuberculosis in infected animals, a 1/100 to 1/1,000 dilution of old tuberculin gave very satisfactory results 4 to 5 weeks after infection. On the basis of the tuberculin reaction and post-mortem examination of animals infected by the intraperitoneal route, it has been found that Coonoor guinea-pigs are, on the whole, more resistant than Kasauli or Madras guinea-pigs. The Amritsar guinea-pigs probably did not come from a controlled source of breeding and were found to be the least resistant, but the results were not uniform. It was also found that female guinea-pigs, in a fairly advanced stage of pregnancy, were much more susceptible than male guinea-pigs or non-pregnant female guinea-pigs. Animals from the above-mentioned sources will have to be injected by the intramuscular route to arrive at more clear-cut results.

Histopathological examination.—On histopathological examination, the lesions could be divided into the following categories :—

1. Lesions resembling those of sarcoidosis, i.e. reticulo-endothelial hyperplasia, presence of giant cells, absence of lymphocytes and of caseation.
2. Reticulo-endothelial hyperplasia only.
3. Reticulo-endothelial hyperplasia + necrosis.
4. Reticulo-endothelial hyperplasia with either necrosis or caseation.
5. Reticulo-endothelial hyperplasia + lymphocytic zone.
6. Persistence of polymorphs for long periods in the lesions.

The preliminary experiments carried out show that two factors are involved in the production of different types of lesions, one is the nature and the dose of the strain injected and the other is the different response of guinea-pigs from different areas. Caseation was found to be more or less absent in tubercular lesions in Lucknow guinea-pigs, whereas it was occasionally encountered in Bombay or Kasauli guinea-pigs. The lesions in the liver specially, and sometimes in the spleen, resembled those of sarcoidosis in a number of cases. The lymphocytes were either scanty or absent in tubercular lesions in most of the organs, there were fairly often encountered only in the lesions in the omentum.

Cultural examination.—The preliminary work shows that tubercle bacilli of low virulence cannot be recovered from organs on culture after several weeks. The organs which do not show any definite tubercular lesions on histopathological examination also reveal the absence of bacilli on cultural examination. The bacilli can be recovered from those organs which show definite tubercular lesions provided the number of bacilli injected is fairly large. More work on the subject is in progress.

VENEREAL DISEASES

1. Inquiry on treponemal agglutination test (TPA test) for syphilis under Dr. C.W. Chacko in the Venereal Diseases Laboratory, Government General Hospital, Madras.

Principle of the TPA test.—A specific antigen in the form of a saline suspension of heat-killed virulent *T. pallidum* (Nichol's strain) extracted relatively tissue-free from rabbit testis syphiloma, is agglutinated by an antibody in syphilitic sera.

Material and methods of preparation of the treponemal antigen and the TPA-test technique and the test-after-absorption-technique have been essentially the same as had been described in the progress reports on the inquiry on September 1954 and 1955 (*vide* I.C.M.R. Technical Report of the S.A.B. for the year 1954-55).

The techniques of the TPA test on C.S.F.—The specimens of C.S.F. tested were from those received from the V.D. Clinic for routine investigations for syphilis. The TPA test was done as an additional parallel test. The C.S.F. was inactivated for 15 minutes at 56°C. and for 5 minutes at 56°C. when tested 4 hours after the initial inactivation.

The TPA antigen was of the same quality as that used in the tests on serum specimens.

The technique of the test proper on C.S.F. specimens, was the same as on the serum specimens except that the C.S.F. was not initially diluted with E.D.T.A. one in 20 in the qualitative tests as in the tests on sera, and in the quantitative test doubling serial dilutions were done with normal saline instead of E.D.T.A. 0.1 c.c. of the TPA antigen was mixed with 0.1 c.c. of C.S.F. and/or its dilution, in a Kahn test-tube and the mixture incubated at 37°C., in a water-bath overnight (18 hours). A drop of the incubated mixture was examined in the darkfield of the microscope for clumping. The titre was expressed as the last dilution which showed definite clumping. A known positive C.S.F. in dilutions and negative C.S.F. were used as controls in each batch of tests.

Technique of testing the stability of the TPA antigen.—The TPA antigen was prepared as described in the methods and a sample of 1 c.c. from each batch, as and when prepared, was preserved in sealed ampoules in the refrigerator 4°C. to 5°C. At the end of about 12 months, parallel quantitative TPA tests were done using these different batch of antigens, preserved for varying months, and a fresh batch of antigen, on known positive and negative specimens of sera. The degree of diminution in titre, if any, was noted to check on the stability of the TPA antigen.

The inquiry was started in April 1954. The TPA test for syphilis has been performed parallel with the VDRL, the WR and the Kahn tests on 1,205 sera, which included 392 specimens from cases in various stages of syphilis, 56 specimens from non-syphilitic venereal diseases, 580 from non-venereal abnormal conditions, 177 from apparently normal persons. On analysis of the results, the sensitivity of the TPA test in syphilis appeared to compare well with that of the routine standard tests for syphilis. As regards specificity checked on normal cases, and non-syphilitic abnormals, the TPA test did not appear to possess any particular advantage over the standard tests. 189 specimens of sera from the syphilitic group reactive to both the VDRL and the TPA tests have been investigated with the test-after-absorption technique. 181 of them became negative to the VDRL test and continued to be reactive to the TPA test after absorption. The remaining 8, which continued to be reactive would need explanation under the circumstances in which 2 distinct agglutinating antibodies are presumably present in syphilitic sera.

Thirty-one specimens of sera from the non-syphilitic group reactive to both the VDRL and the TPA tests and probably biologically false positive in nature, have been examined with the result that 19 of them

became negative to both tests and 12 of them became negative to the VDRL test and continued reactive to the TPA test.

The 19, included 18 from non-syphilitic abnormal conditions, where no definite diagnosis could be established, and one case of leprosy. The 12 sera consisted of 11 specimens from persons suffering in miscellaneous diseases and 1, where a definite diagnosis could not be established. Syphilitic infection has been excluded from these as far as practicable. If the TPA test done by the test-after-absorption technique, is to be considered truly specific, these 12 and 31 cases would need explanation. It is considered that the number of specimens investigated in this category of biological false positive reactions at the present stage of this inquiry is not adequate enough to draw any conclusions as to the specific use of the TPA test. It is planned to confine the test to this category in future so that a conclusion may be definitely drawn.

The TPA-test technique has been adapted for examination of the cerebro-spinal fluid. 361 specimens which included 78 from non-syphilitic conditions, 237 from non-neurosyphilitics and 46 from neurosyphilis cases were tested in parallel with the VDRL and the Kolmer complement-fixation test for syphilis. The analysis of the results with reference to the sensitivity and specificity of the TPA test in C.S.F. did not show any particular advantage for the TPA test over the standard test.

As regards the stability of the TPA antigen preparation, quantitative sensitivity tests on syphilitic sera with the TPA antigens preserved at refrigerator (5°C.) condition it showed that the antigen would stay stable up to 6 to 12 months.

During the period under this report, the Nichol's strain of *T. pallidum* has been transported in rabbits and established in the Pasteur Institute, Coonoor, and Pathology Department of the Grant Medical College, Bombay. Technical staff from the above Institute, have been trained in the isolation and maintenance of this standard strain in rabbits and in the techniques of the tests for syphilis using virulent treponemal antigen. These institutes should be able to take independent investigations in this field in future.

The limited quantity of the TPA antigen suspension that could be prepared from a rabbit, namely about 5 c.c., has limited the experimental investigations into the various aspects of the TPA test, other than the comparative evaluation of the sensitivity and the specificity of the test. It is planned to confine the investigation into the special experimental aspects during the rest of the year, and submit a final complete report of the value of the TPA test. It has been proposed to continue the inquiry during 1957-58 on modifications of the TPA test reported recently by American workers under the name of Treponemal Immune Adherence (T.P.I.A) test or treponemal complement fixation (T.P.C.F.) test for syphilis.

2. Study of incidence and relationship of prenatal and neo-natal syphilis abortions of still-births, neonatal deaths and congenital syphilis and results of treatment with penicillin at various stages under Dr. B.B. Gokhale at the B. J. Medical College, Poona.

1,612 cases were studied antenatally; both clinically and serologically. 189 out of these were serologically positive. Thus, the S.T.S. was positive in 11·3 per cent. cases. Seventy of them came to the hospital for delivery. Their off-springs are beings observed.

Total number of admissions as in-patients has been 2,764.

2,514.....Labour cases with live births.

201.....Abortions

54.....Still births.

263 mothers out of 2,514 that gave birth to live babies were serologically positive. Therefore, the percentage of serologically positive mothers is 10·5. Forty-one out of these were already treated antenatally at a period varying between 21 and 37 weeks of gestation; majority being treated between 28th and 36th weeks of gestation. Fifty-five seropositive mothers were treated after delivery along with their babies (these were admitted as unregistered emergency cases). All the treated cases are being observed.

Out of 201 cases of abortions, 30 were serologically positive. Out of these 13 aborted before 16 weeks; and 17 after 16 weeks. Out of 13 that aborted after 16 weeks 3 were primi-paras. There were also 3 primi-paras amongst 17 that aborted after 16 weeks. Twenty-seven products of conception from 201 cases of abortions were examined histologically; 4 of sero-positive cases and 23 of sero-negative cases. No conclusions can yet be drawn as the number of cases studied is small.

Obstetric histories of 1,922 cases were studied. These women had 7,355 conceptions; 949 of them were sero-positive and 7,355 sero-negative. Total number of abortions out of these conceptions was 477; 81 from sero-positive cases and 396 from sero-negative cases. Percentage of abortions in sero-positive group comes to 8·53 and 6·18 in the sero-negative group (Percentage of pregnancies in the age group of 15 to 20 years has been 7·16 and 8·91 in sero-positive and sero-negative cases, respectively. It has also been observed that there are proportionately more young mothers in the sero-negative group. Therefore, the greater percentage of abortions among sero-positive group could not be due to their youth or immaturity). The differences between the percentages of abortions in the two groups is $(8. - 536 \cdot 18) = 2 \cdot 53$ per cent. This difference is highly significant, being 2·73 times the standard errors which is 0·86. Thus, the rate of abortions in sero-positive cases is significantly higher than in the sero-negative group.

Fifty-four women delivered still-born babies. Fifteen of these were serologically positive. We could do post-mortem examinations in 27 cases; mothers of 7 of these were sero-positive.

Placentæ and babies in all cases are weighed at birth. The ratio between the babies' weight and the placental weight of babies born of serologically positive mothers had been 1:5.782.

The ratio of placental and babies' weight in equal number of sero-negative deliveries of group of mothers of same age and parity has been 5.213. There is no significant difference between the ratios of the two groups.

Thirty placentæ were examined histologically. Out of these 14 were from sero-positive mothers. Nothing abnormal could be detected in 10 of them. Out of the remaining four.—

One showed infarcts,

one showed fibrosis,

two showed histopathological picture of syphilis.

Five X-ray pictures of long bones of the babies of serologically positive mothers have been taken. Two of them were treated antenatally at 32nd week of gestation. The X-ray pictures are normal in both the cases. X-ray pictures of 2 babies of untreated sero-positive mothers are normal and of one shows syphilitic changes.

Cord blood of off-springs of 12 mothers serologically positive by three tests, viz. VDRL, K.T., W.R. was examined at delivery, also by above three tests. None of these mothers was treated antenatally as they came to the hospitals as emergency cases.

Seven mothers were positive by all three tests. Remaining 5 mothers have been positive by K.T., and VDRL.

One baby was positive by all three tests; and 7 were positive by VDRL test only. Out of the remaining four, three were negative by VDRL and W.R. but positive by K.T. and one was negative to all three tests.

Cord blood of babies of seven mothers treated antenatally (because their blood was positive by K.T. and VDRL) was tested by three tests, viz. VDRL, K.T. and W.R. One was positive by VDRL and W.R. Three were positive by VDRL and the rest were negative by all three tests.

Results seem interesting and further study is indicated.

3. Inquiry on the isolation and establishment of virus of lymphogranuloma venereum (LGV) in the embryonated chicken egg and the preparation of the Frei-antigen from it under Dr. C. W. Chacko at the Madras Medical College, Madras.

OBJECT AND SCOPE

It is aimed to isolate a local strain or strains of the virus of LGV from human bubo pus of LGV cases available at the Madras Venereal Diseases Clinic, identify, and establish the same, by maintaining in artificial serial sub-culture in the yolk sac of embryonated chick eggs. When the isolation of the virus proves successful, the object is to prepare Frei-antigen from the infected yolk sacs. Frei-antigen of human bubo pus

origin is available in too limited quantity to meet the great demand for it in the country. This pilot study would help to prepare, on a large scale, Frei-antigen of chick embryo yolk sac origin in the country to meet all the demand for it.

MATERIALS

The selection and clinical diagnosis of the cases for the source of virus are done by the V.D. Clinic.

Five to six days old incubated fertile eggs, of both special and country breed origin are used for the isolation in egg yolk sac. Swiss albino mice, weighing about 15 g. to 18 g. and about 30 days old are chosen for the intracerebral isolation of the virus.

METHODS

Isolation and maintenance of the virus from human bubo pus : (a) *In mice.*—The pus is aspirated aseptically from the bubo of Frei test positive lymphogranuloma patients.

First, direct smears of the pus are made and stained with Gram Castaneida, Macchiavello and Giemsa stains and are examined for any bacterial contamination and for the virus bodies. Sterility for aerobic and anaerobic organisms is done at the same time. Frei-antigen is prepared from the samples according to standard methods and 'reverse Frei test' is done to confirm its LGV origin. If the Gram stain does not show any contamination, an aliquot part of the pus is diluted 1 in 5 with sterile nutrient broth of pH 7.0 and inoculated intracerebrally in 0.30 c.c. quantities into at least 6 lightly ether anaesthetized Swiss albino mice of about 30 days old, using 1 c.c. tuberculin syringe and 1/4 inch BD 26 needle, puncturing the head just above the level of eyes. The animals are kept under observation for a maximum period of 10 days. Even if no symptoms, such as ruffled fur, loss of weight and a hunched back gait are seen due to the specific infection, the brains of the mice are removed aseptically and impression smears of the brain are made and stained with Macchiavello, Castaneida and Giemsa stains and examined for the initial and or elementary bodies of the virus. Whether the virus is seen or not, the brains are ground well, emulsified with sterile broth of pH. 7.0 using 1 c.c. of broth per brain, and the emulsified material re-inoculated intracerebrally in another set of 6 mice in the manner explained above. Three blind passages are made before the experiment is given up.

(a) *In the yolk sac of the embryonated chicken eggs.*—Six-day old incubated eggs are chosen for the primary and the subsequent isolation and the maintenance of the virus in the yolk sac of chick embryos. The eggs are candled to ensure live healthy embryos and air sacs are marked off. A drill hole over shell at the end of the air sac is made but not through the shell membrane. A 1 in 5 dilution of the pus is made in broth containing 1,000 units streptomycin per 1 c.c. After keeping this suspension in the cold for half an hour, 0.2 c.c. quantities of this material are inoculated into 6 eggs through the yolk sac using 1 c.c. tuberculin syringe and 1/8 inch BD needle. The eggs are candled twice a day. The embryos moribund or dead 48 hours after inoculation into the yolk sac,

the eggs are opened, and the yolk sac collected. Smears are prepared from the yolk sac, stained with the four stains and examined for the virus. If the virus bodies are seen or not and if not bacteriologically infected, a 10 per cent suspension of the yolk sac is made in nutrient broth containing 1,000 units of streptomycin per c.c. of broth and inoculated through the yolk sac into another six normal embryos by the yolk sac route. The embryos which had not died by the 5th day are also harvested and treated likewise. Three blind passages are made before the experiments are given up.

Notes on the inquiry up to the end of September 1956.—The inquiry was started on 1st April 1956.

During the period under review efforts were first directed to establish a standard strain of the LGV and or the Psittacosis virus in the yolk sac of embryonated chick eggs. To this end separate yolk sacs infected with the established standard strain of LGV and Psittacosis virus were transported by air in screw-cap bottles in vaccum jar packed in ice from Nagpur Medical College through the courtesy of Dr. Shrivastav. The Psittacosis virus was established but the LGV strain could not apparently be due to bacterial contaminants interfering with its isolation. The Research Assistant was trained and given practice in this type of virus culture work using the Psittacosis strain which is being maintained successfully in serial subculture in the yolk sac. A second attempt to establish a standard LGV strain from a lyophilized sample of it received from Nagpur has been a failure again, due to contaminants in spite of aseptic precautions. Another standard strain (KAM) in lyophilized state was received on 31-7-1956 from the Pasteur Institute, Paris, through the courtesy of Dr. Lepin and attempts are being made to revive it in mouse brain and embryonated chick eggs and then maintain it in the eggs.

Isolation and maintenance of the virus from human bubo pus in mice and in embryonated chicken eggs.—Having gained experience with culture of the Psittacosis virus, preliminary attempts have been made to isolate a local strain of the LGV virus. Six samples of bubo pus from clinical cases of LGV were used. The material in each case was inoculated into white mice intracerebrally and into embryonated chicken eggs according to the techniques described. We have failed to infect the mice and isolate the virus intracerebrally in all the samples. In the eggs, however, although we have failed in the five samples of pus, from the sixth sample, there had been three deaths by the 5th day of inoculation among 9 embryos primarily inoculated and the yolk sac has shown virus-like bodies on staining. At the moment they are being sub-cultured and studied to confirm the possibility of success in the isolation of the local strain.

Frei-antigen was prepared according to Frei's old method from all the 6 samples and tested for potency in known cases of LGV and normal person. Only two, including the sixth specimen, was found potent and only one of it has possibly initiated specific infection in the chick embryo. Once a standard and/or local strain of LGV virus is successfully established and maintained in yolk sac of embryonated eggs, efforts will be directed to prepare Frei-antigen of yolk sac origin.

ENVIRONMENTAL HYGIENE AND SANITATION

1. Inquiry into the effect of sewage treatment on survival of tubercle bacilli in sanatoria sewage under Dr. T.R. Bhaskaran at the All-India Institute of Hygiene & Public Health, Calcutta.

Increasing number of sanatoria have to be built in this country to cope with the problem of treating tubercular patients. Workers in different parts of the world have demonstrated the presence of tubercle bacilli in waste water, sludge and effluents from tuberculosis sanatoria. It is also felt that waste water from slaughter houses, where diseased animals are killed, may contain tubercle bacilli. Unless the wastes are properly disposed, this will create public health hazards. Work on this problem was, therefore, undertaken to determine suitable methods for recovering tubercle bacilli in sewage and determine the efficiency of different treatment method for the safe disposal of effluent and sludge on land or in water.

A number of sewage samples from sanatoria at Jadavpur and at Kanchrapara, near Calcutta, was collected and examined for the presence of tubercle bacilli by microscopical, cultural and animal inoculation methods. All these samples showed the presence of tubercle bacilli. In some instances, sets of effluent samples at different stages of purification, sedimentation, trickling filter treatment, were also examined in addition to the raw sewage. These also contained tubercle bacilli. It is, therefore, concluded that under the existing conditions, the treatment plants are not effectively removing tubercle bacilli from the final effluent, although the efficiency of purification as judged by B.O.D. ranged from 80 to 95 per cent.

More samples of sewage will be examined from sanatoria, as well as from slaughter houses. Primary and secondary sludge from treatment plants will also be examined. The possibility of quantitative estimation of tubercle bacilli at various stages of treatment, using dilution principle, will be explored. Suitable methods which will ensure complete removal of tubercle bacilli from these samples will also be investigated.

2. Industrial wastes disposal and water pollution research unit under Dr. T.R. Bhaskaran at the All-India Institute of Hygiene & Public Health, Calcutta.

Proper sanitary treatment of liquid wastes produced from industries has proved a difficult problem to the health authorities in different parts of the country. Water-pollution problems arising out of them are of serious concern to the local and State authorities in certain regions. One of the urgent problems facing the health authorities and industries in Uttar Pradesh and Bihar States relates to the proper treatment and disposal of liquid wastes from sugar factories, distilleries and tanneries. Work was, therefore, undertaken by the Unit with a view to find out suitable methods of treatment and disposal of these wastes.

The Unit has continued research work on treatment of distillery wastes during the year. Laboratory experiments on different methods of treatment of distillery wastes have been carried out. The wastes have been treated in a small laboratory scale trickling filter designed for this purpose. The results obtained show that the efficiency of purification in an ordinary trickling filter is low and that this method of treatment may not be economical in practice. Anærobic digestion of the wastes prior to biological treatment has been tried, but its efficiency is also very low. Further work on predigestion of wastes under different conditions is now in progress. Aeration of the wastes with sewage shows that the wastes attain a viscous consistency after æration for 72 hours.

Treatment of distillery waste with 2 per cent lime followed by flocculation and sedimentation for one hour was found efficient for removal of about 40 per cent putrescible organic matter (B.O.D.). If a sufficiently large water course providing 1 in 200 dilution is available, the effluent after lime treatment may be disposed of without creating insanitary conditions. The effluent may also be diluted suitably and used for irrigation if sufficient land is available for the purpose. Based on the laboratory findings, an experimental pilot plant has been constructed at Messrs. Bengal Distilleries, Konnagar, Calcutta, for partial treatment of distillery wastes. The pilot plant consists of a reservoir, capacity about 250 gallons, a motor pump and a hopper bottom liming tank of about 180 gallons capacity fitted with a hand operated flocculator. Observation on this plant will be made during the next few months.

Another pilot plant for treatment of sugar wastes has also been constructed at Bhita Sugar Factory near Patna (Bihar) and observations will be made in the ensuing cane crushing season. This plant has been designed to handle about 1,000 gallons of sugar wastes per day.

Work on the use of algae for treatment of wastes was also carried out. An apparatus has been designed for the study of different species of algæ under controlled conditions of light, temperature and nutrients in order to study the oxygen donating power of the different species. This equipment is now being fabricated and assembled by a scientific instrument manufacturer in Calcutta. Work on standardization of a suitable culture technique for mass cultivation of the different species of algae for experimental purposes is also in progress.

The field units for Uttar Pradesh and Bihar States have been established in Lucknow and Patna, respectively, to carry out waste survey in the respective regions. Suitable schedule forms have been worked out for collection of data on different type of wastes. The personnel of these units were given training for 1 week to 6 weeks periods at the All-India Institute of Hygiene & Public Health, Calcutta, in the methods of waste survey, analysis of wastes and other related problems before they took up work in the field units. A laboratory has also been organized in each of these units to carry out analysis of the wastes and other work in connection with the survey. The Lucknow unit has completed survey of the tannery waste in the State. The Patna unit has completed the waste survey in one of the three zones in the Bihar State.

Regional Committees have been established in the two States to review the work of the field units from time to time and to co-ordinate

the activities of the units with the other programmes in this field in the different States.

3. Study on the maintenance of tubewells in the rural areas of Bihar under Shri K. N. Rohatgi, Superintending Engineer, Public Health Engineering Department, Bihar, Patna.

The work of the inquiry started late owing to the late receipt of sanction of the staff. The spade work for starting the inquiry on the right lines has, however, been completed.

The sites of all the 290 tubewells, under study, have been visited and got familiarized by the Laboratory Assistants.

Each Laboratory Assistant assisted by a peon has to go round the tubewells in his jurisdiction for at least 25 days in a month, observe the condition of each and note down the same in the data sheet of the tubewell concerned.

Ten tubewells, sunk during 1951-52, with mesh type strainers which have been found to be choked up, are being re-sunk, half the number with mesh type strainers and the other half with slotted type of strainers; three of the latter will be jacketted and two will be all brass ones. This is being done to study the performance of the different types of strainers under similar conditions of soil and use.

4. Study of the different types of latrines used in rural areas under Professor F.K. Erickson at the All-India Institute of Hygiene & Public Health, Calcutta.

Disposal of human excreta presents a major problem to-day in the rural areas of India. Latrines are practically absent in these areas and a few that exist are very unsatisfactory. An effective sanitation programme in the rural areas depends primarily on the evolution of a satisfactory latrine and acceptance of the latrine by the people. Conditions vary widely in different regions of the country. Social structure, habit, custom, tradition and geological formation differ from place to place. Environmental Hygiene and Sanitation Advisory Committee recommended in 1955, that a study be undertaken in the different regions of this country with the object of evolving satisfactory latrines for these regions. A study was to be carried out in the States of West Bengal, Travancore-Cochin, Bombay and Punjab in the first instance.

Work done.—Successful implementation of this inquiry can only be had through the co-operation of the public health engineering departments of the States concerned. State Public Health Engineers of Bombay and Travancore-Cochin have agreed to give all assistance in the implementation of the scheme in their respective regions. The Unit in West Bengal is located within the Singur Thana Health Centre which operates under the direct supervision of the All-India Institute of Hygiene & Public Health. Staff for these units are being recruited.

A study of septic tank as a means for disposal of human excreta is underway at Singur. Observations with respect to the performance of the septic tanks and also on the disposal of effluents by soakage pits and tile fields are in progress.

INDUSTRIAL HEALTH

1. Industrial Health Research Unit under Dr. M.N. Rao at the All-India Institute of Hygiene & Public Health, Calcutta.

A. INDUSTRIAL HYGIENE

Air pollution.—The work started last year in Calcutta is continuing on a pilot scale. Samples of dust fall are collected from ten representative centres every month in special jars put for that purpose on the roof of buildings. The monthly average of dust fall was found to vary from 30.9 to 111.11 tons per sq. mile over a period of last twelve months. The insoluble portion of the dust fall is analysed for its ash and volatile contents. Samples of air are analysed twice every week for SO_2 , NH_3 , NO_2 and aldehyde contents from one centre. Of these gases the concentration of SO_2 was found to vary between 0.01 and 0.15 ppm., and NO_2 between 0.02 and 0.29 ppm., NH_3 between 0.08 and 0.87 ppm., and aldehyde between 0.05 to 0.39 ppm. Though the site of collection of the gases is the premises of the All-India Institute of Hygiene & Public Health which is centrally situated in the city of Calcutta, yet it is not possible to draw any conclusion on the basis of the results from a single centre. A more elaborate study of the gases collected from a number of representative centres of the city is being planned.

B. INDUSTRIAL HEALTH-PHYSICS

(i) *Radiation hazards.*—The investigations on the radiation hazards conducted by the Unit in medical X-ray installations have stimulated considerable interest amongst the workers using radiation for medical or research purposes. The Unit has been approached by governmental and non-governmental agencies (e.g. the Radiology Department, Calcutta Medical College) for technical services, viz.—

- (a) Advice on the continuation of radiation monitoring; and
- (b) assistance in the implementation of proper protective measures, such as design of lead shields, limitation of exposure time, etc.

Future extension of the technical activities as a routine health physics service with additional equipment is proposed. The up-to-date results of the investigations are ready for publication.

(ii) Instrumentation and techniques :

(a) *Construction of a new bicycle ergometer.*—The need for a more reliable bicycle ergometer has been felt for a long time in this Laboratory. An ergometer in which different, fixed light and moderate exercise levels at constant rates can be performed by Indian volunteers has been designed and constructed by the Unit. This set-up is essentially an ergometer with a dynamo brake i.e., a bicycle coupled to an electrical generator, which acts as the brake. Five uniform steps of exercise grades up to about 90,000 ft. lb. per hour are made available by suitable resistances in the external circuit of the dynamo. Arrangements for speed synchronization and measurement of electrical output are also provided. Detailed physical and physiological tests of performance of this ergometer have been carried out for standardization.

(b) *Urinary chloride determination.*—The techniques developed for the study of salt concentration in sweat have been extended to urine also. The specific gravity, specific conductivity and the salt concentration of 28 samples of urine from normal healthy individuals in summer and 25 similar samples in winter have been determined. It is found that there is a good correlation between these physical properties and chloride concentration in urine. The usefulness of this physical method for field determination of urinary chloride has been demonstrated. The temperature co-efficient of the specific conductivity has also been determined and the importance of the temperature correction factors emphasized.

(iii) *Thermal environment.*—Considering the heat dissipation of the human body at work as an essentially physical problem, a preliminary study of the body heat content and evaporative cooling has been made. The investigation of the relation between the rate of sweating (an index of evaporative cooling) and rectal temperature (an index of body-heat content) during physical exercise at a constant rate on a bicycle ergometer has been carried out on five volunteers. Five to six experiments were done on each of the volunteers once a fortnight from October to February. In each experiment the subject was weighed nude every 10 minutes on an accurate lever balance and the simultaneous rectal temperature recorded with an electrical thermometer. The climatic conditions prevailing during the experiment, as specified by the effective temperature, were also determined by the usual methods. The rate of sweating was correlated to the corresponding rectal temperature.

In general it is found that—

(a) In the hot-humid climate (effective temperature greater than 70°F.) the rate of sweating bears a positive correlation to the rectal temperature and, therefore, to the body heat content.

(b) At lower effective temperatures the regression line between the sweating rate at work and the rectal temperature flattened out, thereby indicating that the sweat loss remained constant although the body heat content increased.

(c) At the lowest effective temperature attained—less than 67°F.—the regression lines have even shown a negative slope, i.e. the rate of sweating decreased with the increase of the heat content of the body.

Extreme individual variations in the heat regulating mechanism seem to exist. Of the five subjects, one showed exactly the opposite behaviour to that described above. Physiologically, these experiments corroborate the fact that during the cooler season radiation as compared to evaporation assumes a greater importance in the heat dissipation from the body. The present data are, however, not conclusive enough to make any deductions regarding the changes in the heat dissipation of the body during work in winter. Further experiments with improved techniques (e.g., greater accuracy of sweat loss determination with a more sensitive human weighing device and greater constancy of work rate on the ergometer) are planned.

C. INDUSTRIAL PSYCHOLOGY

Study on industrial tension.—Two groups of workers were selected from the same working population with similar characteristics. The workers of the foundry section formed the experimental group for the Action Research Programme and the workers of the machine shop constituted the control group. Pre-experimental attitude survey of the two groups *plus* others in the same factory was reported at the last annual meeting. The six supervisors of the experimental department took part in a nine-day supervisors' orientation programme emphasizing on Employer-State-Workers' relationship. Similarly, after a period of three months, 21 selected workers from the same department participated in a five-day workers' orientation programme. After training, the participants went back to their old jobs and radiated their newly acquired experiences among the fellow workers through their daily informal meetings. On the fourth month from the termination of the training programme, post experimental attitude survey in both the departments was taken up to compare the effective value of group participation on workers' morale and efficiency. The same multiple choice type of weighted attitude scale reported previously was used in both the cases.

From the analysis of the results and comparison of the two groups, the following conclusions are drawn :—

(1) Interpretive group discussion together with the use of audio-visual technique is found to be an effective method of improving participants' mental dispositions. A highly significant 't' test value of 20.5206 shows the difference between the pre- and post-experimental attitude test scores of the group under experiment. On the other hand no such significant change is observed in the attitudinal disposition of the control group.

(2) Our programme also seems to improve the relationship between the different levels of personnel in the experimental group during the post experimental period. No such change was observed in the control group.

(3) Significant improvement in per capita production rate of the experimental group at 0.01 level is observed. A reduction of per capita rejection of the finished product in the experimental department was also noted after the training programme.

Conclusions from this study of the improvement of social climate in improving the quality and quantity of the industrial products through such training programmes are of national importance.

D. SOCIAL SCIENCES

Medico-social follow-up.—The Mass Chest x-ray Unit at the All-India Institute of Hygiene & Public Health, Calcutta, has surveyed 35 factories employing 46,315 workers. By preliminary miniature X-ray and subsequent large size repeats, 385 workers were found to be radiologically positive. Actually, 162 radiologically positive cases could, however, be contacted by the Medico-Social worker of the Unit during this

year. Sixty-seven amongst them were contacted for the first time. Most of them were contacted in their homes and some in factories themselves. The Medico-Social Survey during the year was concentrated on intensive interviewing with the following objectives :

(1) To have a first hand appraisal of the environmental conditions at home and ascertaining special predisposing factors or personal habits, responsible for the infection.

(2) To analyse the factors, if any, why the workers are not continuing the treatment recommended by this and other agencies.

(3) To contact the managements of some of the works with regard to help in treatment programmes of the factory men and rehabilitation problems of workers.

(4) Health education of the workers and the home contacts with regard to the communicability of the disease.

E. APPLIED PHYSIOLOGY

(i) *Differential lung volume in health.*—This work, initiated to establish the norms for Indian population is expected to be completed by the end of the year. A total of 31 males and 70 females has been finished up-to-date, and a total of 180 is the target to give 12 subjects in each age group. The Table summarises the data up-to-date.

(ii) *Differential lung volume in disease.*—A total of 21 patients suffering from diseases, such as tuberculosis and asthma, was also investigated with reference to their lung volume and lung capacity. The group being heterogenous is not comparable to the norms. The Unit was giving technical service to the hospital surgeons and physicians.

(iii) *Seasonal variation of B.M.R.*—This study is completed during the year and the data analysed. The detail is expected to be ready for publication before the end of this year.

F. INDUSTRIAL MEDICINE

Excretion of coproporphyrin in health and diseases.—The results of an investigation on urinary coproporphyrin excretion in some tropical diseases and lead poisoning were reported in 1953. It was found, in that study, that besides lead poisoning, excess porphyrin is also excreted in malaria, kala-azar, filaria, etc. This study was, however, qualitative in nature without giving the exact amount excreted.

With a view to find out the actual amount of coproporphyrin excreted in health and in various diseases referred to above, the present investigation has been undertaken. Uptil now spot samples of urine from 20 healthy volunteers have been analysed. The concentration of porphyrin in the urine has been found to lie between 0.10 and 0.3f mg/litre.

The total amount of coproporphyrin excreted in 24 hours urine has been found in two cases to be 195.8 mg. and 123.9 mg.

The work is in progress.

(Lung volumes recumbent posture at 'BTSP')

Sex	Number of subjects	Age, years	B.S.A. m ²	I.R.C. L/m ²	T.V. L/m ²	E.R.C. L/m ²	V.C. L/m ²	R.C. L/m ²	F.R.C. L/m ²	T.C. L/m ²	$\frac{R.C.}{T.C.} \times 100$	$\frac{F.R.C.}{T.C.} \times 100$
Males	Mean	28.6	1.62	1.23	0.40	0.53	2.22	0.65	1.19	2.87	22.6	41.1
	31											
	Range	18-45	1.38-1.88	0.69-1.76	0.24-0.81	0.13-0.90	1.69-2.79	0.39-1.04	0.82-1.97	2.31-3.83	14.1-27.2	22.1-56.3
Females	Mean	29.5	1.43	0.95	0.316	0.353	1.36	0.55	0.91	2.21	24.9	40.8
	70											
	Range	15-49	1.10-1.76	0.61-1.50	0.207-0.729	0.095-0.666	1.22-2.28	0.26-0.86	0.48-1.46	1.52-2.91	12.1-35.5	28.3-52.8

2. A study into incidence of pneumoconiosis in central Indian coalfields under Dr. K.B. Roy at the Nowrozabad Colliery, Nowrozabad, Vindhya Pradesh.

A Note on the work done.—Any person who has worked in any job at a coalmine which requires his presence in the then for the whole duration of a daily shift of 8 hours, was included in the radiological survey. A total of 135 chest skiagrams of such persons has been taken revealing 11 cases of complicated pneumoconiosis, 16 cases of simple pneumoconiosis and the rest negative.

All patients with complicated pneumoconiosis except one, have worked in underground jobs for more than 20 years; six of them have been disabled by their pulmonary condition which compelled them to give up their job as a miner. Erythrocyte sedimentation rate was found raised to a variable extent in all cases with complicated pneumoconiosis. One case of cystic disease of lungs and three cases of pulmonary tuberculosis were diagnosed radiologically in patients who did not complain of any symptoms.

Conclusion.—Coalminer's pneumoconiosis is prevalent among miners in central Indian coalfields to an extent sufficient to make it a medical and social problem of some magnitude in these areas.

3. Inquiry into absenteeism under Dr. H.P. Dastur, Medical Officer, Tata Industries Ltd., Bombay.

A meeting was held at Tata Mills with the manager and the departmental heads to explain to them the what, why and how of the inquiry. Their consent was obtained to start it. Next, Mr. Y.S. Pandit, Officer-in-charge of the Department of Economics and Statistics, Tata Industries Private Limited, Bombay, was consulted for working out the general outline of the inquiry. It was decided that the start of the inquiry be limited to permanent workers of the Spinning and Carding Departments employing a little over 1,000 workes. Two research workers were selected and they were first advised to emoe among these people and explain to them the work they were going to do and for what. They moved about for a week together with representatives of the Industrial Health Department and talked to groups of workers, jobbers and supervisors and fully explained the aim and perpose of the study. An assurance was given that the data will be treated as confidential and not used for any other purpose.

METHODS OF WORK

The main work consists in interviewing the absentees. For this a committee consisting of the research workers, three representatives of the Industrial Health Department and the Head of the Statistics Department worked out an absentee employee card. This form also includes all the data collected from the interviews.

Another item included in the form is frequency of absences of any worker in any particular month tabulated according to the causative factors. At present 10 such factors have been worked out. They have been codified from A to J.

About 1,800 interviews have taken place. The findings so far have not been statistically analysed. But they suggest certain trends, such as more absences in the night-shift than in the day-shift, increase of absences at the time of change of shifts, increase of absences a day before or after a holiday or after pay-day, in sickness absenteeism from digestive disorders and so on.

4. Inquiry on bagassosis at the Vallabhbhai Patel Chest Institute, Delhi.

Experimental studies on Bagassosis have been started. Instillation of bagasse dust into the lungs of experimental animals produces uniformly a pneumonic change, which is histologically different from that produced by Silica particles of the same size.

Attempts are being made to study the pathogenesis of the acute and chronic lesions. On culture, about a dozen types of bacteria and 4-5 types of fungi have been grown. Identification of these organisms is being attempted. Pure subcultures of these organisms will then be instilled into the lungs to study the pathologic change.

A chemical analysis of the bagasse fibers has also been done. The individual components have been separated and are to be tested separately in experimental animals.

An elaborate dust chamber is under construction for prolonged experiments. The Assistant Research Officer (Clinical Research) has joined recently and will soon undertake a survey of Bagassosis in factories where this condition is a problem.

MATERNAL AND CHILD HEALTH

1. Hæmatological investigations of new-born babies born of anæmic mothers under Dr. R. Dutta Chaudhuri at the Chittaranjan Cancer Hospital and Chittaranjan Seva Sadan, Calcutta

MATERIAL AND METHODS

Total number of studies included 1,439 cases of which 333 were individual mothers and 364 were individual babies. One hundred and forty-two babies which include 86 of the baby cases of last two years, could be only followed-up and that also up to various age periods, 15 cases being upto two years.

The cases studied under the different groups, *viz.* normal mothers and their babies 72 (36+36), microcytic hypochromic mothers and their

babies 48 (24+24). Normocytic anæmic mothers and their babies 78 (39+39). Macrocytic anæmic mothers and their babies 56 (28+28). Dimorphic anæmic mothers and their babies 50 (25+25) and treated anæmic mothers and their babies 28 (14+14) and pre-natal mothers being 71 in number.

Among the follow-up babies, babies of normal mothers were 43 and those of the anæmic mothers were 99. During the follow-up, the nature of the diet, weight of the baby along with the history of illness were recorded carefully.

Bone-marrow smear studies were done on 34 mothers and 18 new-born infants of which 2 were of normal mothers, 6 of microcytic hypochromic mothers and 3 of dimorphic anæmic mothers and 7 of macrocytic anæmic mothers on their 4th day of life.

METHODS AND TECHNIQUES

Methods and techniques of the hæmatological investigations were the same as was mentioned in the Technical Report of the Indian Council of Medical Research (1954) and as described by Dutta Chaudhuri and Chaudhuri (*Jour. Ind. Med. Assoc.*, **8**, (1956)). In this year, hæmoglobin values and the instruments used in hæmoglobin estimation were also standardized against the standard blood samples obtained from London under the scheme of the British Medical Research Council.

Serum iron and iron-binding capacity of the serum:—Method of Davis *et al.* (1952, *Jour. Clin. Path.*, p. 312) was followed.

Estimation of different fractions of serum proteins:

1. *Chemical method*.—Method of Wolfson *et al.* (1948, *Amer. Jour. Clin. Path.*, **18**, p. 723) was followed.

2. *Paper electrophoresis*.—Close strip method. (i) *Buffer*.—Barbiturate buffer solution pH 8·6 u, with ionic strength 0·075. (ii) *Filter paper*.—3 mm. Whatman, 44 mm. × 3 mm. (iii) *Time hours*.—10 hours. (iv) *Potential in volts*.—250, 6 M.A. (v) *Room temperature*.—24°C. to 5°C. (vi) *Dyeing reagent*.—Bromophenol blue solution.

FINDING WITH SHORT COMMENTS

1. There was no significant difference between the hæmatological values of babies of normal mothers and those of the anæmic mothers on the 1st day, and 4th day of their life. The average hæmoglobin values of the mature babies of normal mothers varied from 15·5 g. to 19·8 g. per cent, the average being 17·14 g. per cent. The average value of erythrocytes was found to be 5·1 millions; m.c.v., 106·2 cu; m.c.h.e., 32·4 per cent.; reticulocytes, 5·5 per cent; and leucocytes count 13,400 per cmm. of blood. The average hæmatological value of 66 mature babies of anæmic mothers (microcytic hypochromic; normocytic, dimorphic, macrocytic and treated group) varied as follows: Hb, 16·8 g. to 18·26 g. per cent, erythrocytes 4·96 to 5·31 million per cmm. of blood. M.C.V.—106·5 to 110 cu.; m.c.h.c., 32·1 to 33·6 per cent;

reticulocyte, 4.8 to 5.6 per cent; and leucocyte count—10,500 to 14,300 per cu. mm. of blood on the 1st day of their life. On the 4th day the hæmatological value of the babies of normal mothers and anæmic mothers showed the fall in all values except that m.c.h. and m. of c.h.c.

Hæmatological values of the premature babies of normal mothers also did not differ significantly from those of the anæmic mothers. The average hæmoglobin values of the premature babies of normal mothers were 18.9 g. per cent. which was found to be 1.8 g. per cent. higher than those of the mature babies. All the hæmatological values were also found to be higher than those of the mature ones. The smear of the peripheral blood of the premature babies also showed preponderance of immature granulocytes.

2. Incidence of prematurity and low birth weight seems to be related to degree and types of anæmia in their mothers. 81.6 per cent. babies of dimorphic anæmic mothers and 82.6 per cent. babies of other severely anæmic mothers were premature, whereas only 23.1 per cent. babies of normal mothers were observed to be of lower birth weight than that of normal babies.

3. It seems from simultaneous bone marrow-smear studies of mothers and their babies (2 normal and 16 anæmic mothers) that bone-marrow examination of new-born babies of anæmic mothers indicate the nature of deficiency of hæmopoietic principle in their mothers, whereas the peripheral blood smear had no such finding. Bone-marrow smear of the 7 new-born babies of severe nutritional macrocytic anæmic and dimorphic mothers showed the presence of polychromatic macronormoblasts, giant metamyelocytes, bizarre myelocyte. Three new-born babies of severe microcytic hypochromic mothers showed pyknotic normoblasts in their bone-marrow smear like those of their mothers.

4. *Relation of hæmatological values with the birth weight and gestation period of new-born babies.*—Study of 143 new-born babies showed that hæmatological values (Hb., erythrocytes, hæmatocrit values) of mature babies with gestation period varying from 39 to 41 weeks, did not vary significantly with birth weight of the babies. Average hæmoglobin values of the mature babies weighing between 5 lb. 8 oz. and 8 lb. 15 oz. varied between 16.66 g. per cent. and 17.76 g. per cent. in all weight groups. In premature babies it was, however, observed that hæmoglobin values varied inversely as the birth weight and gestation period.

5. *Hæmatological values and sex of the babies.*—When the sex was considered it might be that there was no significant difference in hæmatological values between male and female babies.

6. *Findings of the follow-up babies of normal and anæmic mothers.*—101 mature babies (36 of normal and 65 of anæmic mothers) were subjected to periodical investigation at various age periods. Fifteen babies only could be followed after 2 years of age. The hæmoglobin values of the mature babies of normal mothers dropped

down to 10.0 g. per cent. on the 3rd month from 17.14 g. per cent. on the 1st day. It then gradually rose to 10.87 g. per cent. at the 6 months and 11.04 g. per cent. at one year of age. After that hæmoglobin again fell to reach the level of 10.26 g. per cent. at the 2 years of age.

It seems that babies of anæmic mothers developed anæmia from 6 months onwards (8.33 g. per cent. of hæmoglobin on average). Four out of 13 cases, however, showed anæmic picture even at their 2nd month. All these babies reached the level of about 7.65 g. per cent. at their 9 months age. It seems that there might be a correlation between the types and degree of anæmia in mothers and development of degree of anæmia in their babies.

The premature babies of both normal and anæmic mothers seems to become anæmic from 4th month onwards. Five out of 10 cases showed anæmia even from 2nd month of their life. If they remain untreated, the anæmia becomes much more marked at their late infancy.

Relation of dietary habits with hæmatological values in late infancy.—Though it is not possible to give any conclusion from the number of follow-up babies in this series (39 only), yet it seems that those who take solid food in late infancy, have higher hæmoglobin values than those who are having milk diet till then, even the babies of anæmic mothers, taking mixed diet, have slightly higher amount of hæmoglobin than those of the babies of normal mothers taking only milk diet.

7. *Hæmatological values in expectant mothers at various weeks of gestation.*—The observation indicates that as the pregnancy advances on and from 25th week, the hæmoglobin level (11.74 g. per cent.) gradually falls in untreated mothers (10.05 g. per cent. on 37 to 40th weeks).

8. Observations on the serum iron and different fractions of protein mothers and their babies were based on 152 cases of which normal non-pregnant women, 40 cases, pregnant women 34 cases, anæmic pregnant women 22 cases and new-born babies 56 (34+22).

Serum iron values of mothers and their babies.—Babies of anæmic mothers seem to have lower serum-iron content than those of the mothers. The average serum iron of babies of anæmic mothers was 68.1 µg. per cent (44 µg. to 104 µg. per 100 c.c. of serum), whereas that of the babies of normal mothers was 105.5 µg. (50.6 µg. to 165 µg. per 100 c.c. of serum). The average serum-iron value of anæmic mothers was 60.3 (14.8 µg. to 91 µg. per 100 c.c. of serum), and that of normal mothers 97.45 (62 µg. to 189 µg. per 100 c.c. of serum).

Serum protein (different fractions) values of mothers and babies.—It seems that there may not be any significant difference between the values of different fractions of protein of the babies of normal mothers and anæmic mothers. New-born babies of normal and anæmic mothers have 4.48 (4.3 to 5.0) and 4.45 (4.1 to 5.0) g. per cent. of total protein in serum, respectively, on average. Average value of alpha beta, gamma globulin of serum of new-born babies of normal and anæmic mothers are 0.37g., 0.75g., 0.65g. per cent. and 0.35g.,

0.45g., 0.56 g. per cent, respectively. The average total protein content of normal expectant mothers was 5.02 g. per cent and that of anæmic mothers 4.8 g. per cent of the serum.

Sera of 4 babies and their mothers were studied by paper-electrophoresis method. The results were more or less similar to those obtained by chemical method. The α_1 and α_2 fractions of the new born babies were slightly higher than those of their mothers.

The data on which these observations were based are not adequate enough to give a valid conclusion. So, it is expected that the continuation of the work will help to come to a definite conclusion in all the aspects dealt above.

2. Inquiry on the oestrogen content of urine in toxæmia of pregnancy with special reference to etiology and prognosis under Dr. S. Mitra at the Chittaranjan Seva Sadan and Chittaranjan Cancer Hospital, Calcutta.

One hundred and fifteen cases of normal pregnancy against 72 cases of toxæmic pregnancy (total 187 cases) have been studied.

The method used, as previously reported, was the modified technique of Stevenson and Marrian for the chemical assay of oestrogen and the fractions from urine. Since 1955, work on column-chromatographic method as suggested by Braunsberg for fractionation of the three fractions of oestrogen has been carried on.

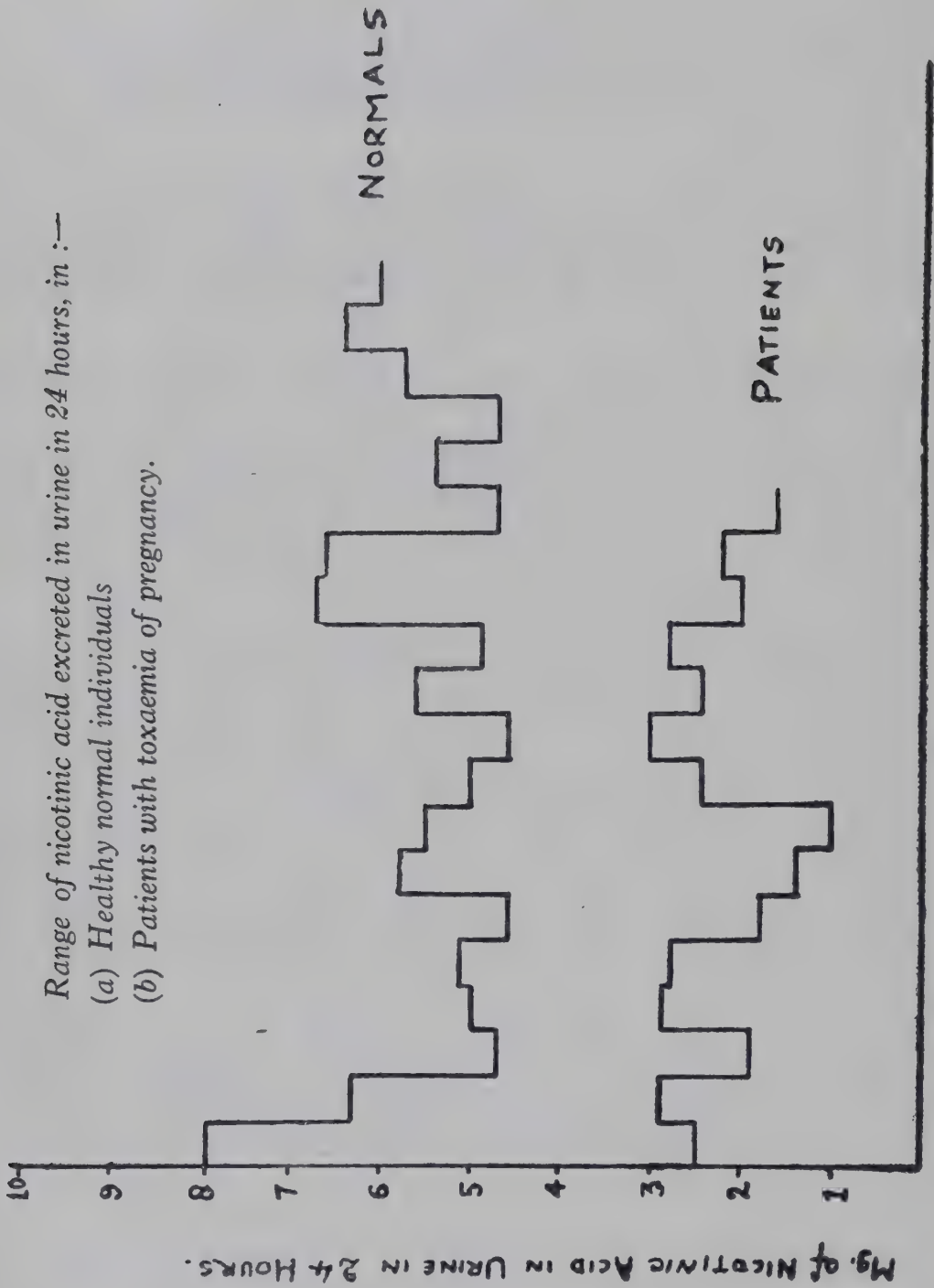
Wide range of variation in the daily urinary output of the oestrogenic hormone in pregnant women, both normal and toxæmic has been observed. This has also been the experience of other workers. This variation is inherent in all hormone assays. This wide range of variation may be due to (i) experimental error, (ii) collection of 24-hour urine, and (iii) general metabolic disturbances.

So, far as oestradiol in toxæmic cases is concerned the excretion rate is slightly lower than that in normal cases. Unlike the total oestrogen and oestriol curve, the level is, however, more or less constant all through the pregnancy without any rise or fall both in normal and toxæmic cases.

So general trend of excretion in toxæmic cases is significantly lower than in normal pregnancy. the oestrone excretion in toxæmic patients is *often higher* than in normal cases, particularly at the 37 to 38 week period. This is significant if it is presumed that in toxæmia a reversible oestradiol-oestrone reaction takes place resulting in low excretion of oestriol.

From the data it might be presumed that the general excretion rate of oestrogen in toxæmia is lower than in normal cases although following a similar pattern.

One is also tempted to suggest that there might be some changes in the intermediary metabolism of oestrogens at the oestrone level in toxæmia of pregnancy.



3. Study of nutritional status of expectant mothers with particular reference to the incidence of toxæmia of pregnancy in Northern Madhya Bharat under Dr. M.K. Lokre at the G. R. Medical College, Gwalior.

MATERIAL

All cases of toxæmias and anæmias that were admitted in the Obstetric Department from July 1955 to March 1956, both months inclusive, were included in the present study. They were divided into three groups, *viz.*,

1. Toxæmias with normal hæmoglobin level ; toxæmia (A).
2. Toxæmias associated with anæmias ; toxæmia (B).
3. Anæmias.
4. 230 cases of normal deliveries were taken as a control.

METHODS

Toxæmia.—Raised blood pressure was the main criterion for including a case in this group. Majority of the cases were unbooked and those which attended the antenatal clinic were seen first time after the 24th week of pregnancy, hence no attempt was made to divide them into pre-eclampsia and chronic hypertensive diseases. These toxæmias were graded into five groups ;

Grade I	Systolic pressure	130 to 139 and/or diastolic pressure	90
Grade II	-do-	140 to 149	
Grade III	-do-	150 to 159	
Grade IV	-do-	160 and above.	
Grade V	Any of the above groups associated with convulsions.		

Anæmia.—A case was called anæmic if the hæmoglobin was below 10 grammes per cent. Hæmoglobin 14·8 grammes per cent was taken as normal. A complete hæmogram was done in anæmic patient.

Nutrition survey.—A nutrition survey was made by investigating the mother's income, diet, nutritional status and certain biochemical analysis.

Gradation of the diets.—Assessment of the diet was made by questionnaire methods. Diets of 230 normal pregnant women at term were analysed. Practically, all of them were vegetarians. These diets were graded into eight categories.

(1) Excellent (2) good (3) fair (4) poor (5) very poor (6) compromised fair (7) compromised poor (8) compromised very poor.

The excellent and good diets corresponded to the standard diets recommended for a pregnant woman at term and that for a non-pregnant woman, respectively. In fair, poor and very poor diets there was a corresponding fall in all the nutrients by 25, 150 and more than 50

per cent of the good diet i.e. they were deficient in energy as well as in protective nutrients. Compromised diets were the diets of the farmers and labourers. In these diets caloric intake of the food was sufficient as in the excellent or good diet, but the intake of protective nutrients was low as in fair, poor or very poor diets. Hence, they were called as compromised fair, compromised poor or compromised very poor according to the intake of the protective foods (Table).

Rating of the diets.—Mean general rating of the diets was done on the lines of Barks method (*Jour. Nutr.* **38**, p. 453, 1949).

All the nutrients were divided into two groups as energy foods and protective foods. Total calories and proteins consumed were also grouped along with the energy foods. Numerical values were assigned to the nutrients as well as the calories and proteins according to the range within which the nutrient fell.—

Excellent 4, good 3, fair 2, poor 1, and very poor less than 1. These numerical ratings were averaged and re-converted to the terminology representative of this average value of the diets (Table A).

RESULT

The aim of this study was to see (i) if an anæmic patient was more likely to develop toxæmia, and (ii) to see what type of nutritional status a toxæmic mother had in comparison to an average pregnant woman at term.

(1) *Relation of anæmia to toxæmia.*—During the first three months of the study, out of 565 total deliveries, 26 were toxæmias (A), 20 were toxæmias (B), and 72 were anæmias. 75 per cent of the toxæmias were of mild type in which the systolic blood pressure varied between 130 and 150. There were 4 cases of eclampsia in toxæmia (A) group. During this period hæmoglobin percentage was done in every case that was admitted in the obstetric department. Hypochromic anæmia was in predominance.

In toxæmia (B) group hæmoglobin varied between 2.99 per cent and 9.89 per cent. The severity of toxæmia showed no relation to the degree of anæmia, nor any affinity to a particular type of anæmia, but from the above figures it was noted that an anæmic mother was four to five times more likely to develop toxæmia.

(2) *Nutritional status. Control (230 cases).*

General health.—On the average, the woman was of small stature with fairly wide hip width. Her weight varied between 90 lb. and 110 lb. and she did not show overt signs of vitamin deficiency.

In 60 per cent of the women the height varied between 4 feet 10 inches and five feet and the most common hip width was 9 to 10 inches. In six out of ten cases in whom the hip width was 10 to 11 inches, the height was above five feet. There were two cases who had hip width between 8 and 9 inches and their height was below 5 feet.

Income and diet.—The majority of the women had low income. Only in 14 per cent the monthly income was Rs. 200 or above. Average family consisted of three to six members.

Roughly one half of the women had good or excellent diets, and in 25 per cent of the women the diet was of compromised type. The rest of the diets were fair, poor or very poor. On the whole, milk was consumed in a small quantity; only twenty-two women who belonged to the excellent diet group took the optimum quantity, i.e. 16 oz. of milk per day. 101 women did not take milk or had only a dash of milk in the tea.

Toxæmias and anæmias (cases collected in the last six months of the investigation).

Parity.—Toxæmia (A) group (toxæmia 57 cases+eclampsia 2 cases) had a higher incidence of primiparas and second paras. Nearly 40 per cent were primiparas and 20 per cent were second paras. Overall incidence of primiparas for the total deliveries (922 cases) was 21 per cent.

Toxæmia (B) group (toxæmia 32 cases+eclampsia 2 cases) and the anæmia group showed a lower incidence of primiparas and second paras. In these groups primiparas were 26 per cent and the second paras varied from 8 to 14 per cent.

General health.—Women from both the toxæmia groups were heavier in comparison to the controls and the anæmic women showed a tendency for low weights. Height and hip width was same as in the controls. Toxæmic women associated with anæmia and the anæmic women showed multiple signs of vitamin deficiencies according to the severity of anæmia.

Income and diet.—Income of toxæmia (A) group closely followed the pattern of the controls, while that of the other two groups showed a higher incidence of low incomes.

In comparison to the controls, the toxæmia (A) mothers had a higher incidence of good and fair diets and lower incidence of poor and very poor diets. Severity of toxæmia did not show any affinity for low grade diets: (1) In both the cases of eclampsia the diet was excellent, and (2) out of nine cases of grade IV toxæmia, one had excellent diet, six were in good diet group, one was in poor diet group and another belonged to compromised fair diet group.

Toxæmic mothers associated with anæmia and anæmic mothers had a higher incidence of low grade diets.

Biochemical analyses:

Serum calcium (micro-burette method).—Common ranges of serum calcium estimations were 8 to 9 mg., 9 to 10 mg., and 10 to 12 mg., per cent Serum-calcium levels did not show any variation according to the severity of the toxæmia, but they varied according to the grade of the diet.

Total plasma proteins (micro-Kjeldahl's method).—In a large number of cases the total plasma proteins varied between 6 and 7 g. per cent. Thirty-nine cases from toxæmia (A) and 24 cases from toxæmia (B) group came under this range. Lower values were obtained from the diets of low grades in both the above investigations.

SUMMARY

1. The nutritional status of the toxæmic mothers with normal hæmoglobin level fared well in comparison to the controls.
2. Toxæmic mothers associated with anæmia had a higher incidence of low-grade diets. Incidence of these cases can apparently raise or lower the number of high or low grade diets or they can mask the nutritional status if the toxæmias are not divided into two groups as has been done in the present investigation.
3. An anæmic mother is four to five times more likely to develop toxæmia than a pregnant woman with normal hæmoglobin level.
4. The diets are graded by mean of general rating.

TABLE

Gradation and mean general rating of the diets.

ENERGY FOODS (E)					PROTECTIVE FOODS (P)					NUMERICAL VALUE		
	Calories	Proteins	Rice, oz.	Dal	Oil, oz.	Ghee, oz.	Sugar, oz.	Milk, oz.	Green veg., oz.	Othe veg. oz.	Fruits, oz.	Meat Fish eggs. oz.
Excellent diet	2,500	80	16	2½	1½	½	2	16	2	6	4	4 of meat fish or 2 eggs E4 P4
Good diet	2,000	60	12	2	1	½	1½	8	2	4	2	... E3 P3
Fair diet	1,500	40	10	1½	1	...	1	4	1	2	1	... E2 P2
Poor diet	1,000	30	8	1	½	...	½	2	½	½	...	... E1 P1
Very poor	Less than above											
Compromised fair	E4 to 3 P2											
Compromised poor	E4 to 3 P1											
Compromised very poor	E4 to 3 P less than 1											

4. Inquiry on nutritional assessment of pregnant women under Dr. B.B. Patwardhan and N.C. Datta at Grant Medical College, Bombay.

The present investigation was undertaken to assess the nutritional status of pregnant women by a combination of dietary intake, medical histories, clinical data and biochemical determination of blood components which are of metabolic and functional importance.

The series consisted of 200 pregnant women selected from patients attending the antenatal department of Bai Motlabai Hospital, Bombay. These patients represent the low-income group of the population.

Age and Parity.—The age varied from 17 to 40 years with 75% of the cases between 17 and 30 years. The parity varied from primipara to IXth. para, with 56 cases belonging to primipara group. Excluding the 56 cases of the primipara group, 144 mothers gave birth to 424 children, an average of 3 children per mother.

Weight and Height.—Majority of the mothers were of short stature. Of the 200 cases, 140 women were below 5 ft. as compared with the mean height of 5 ft-4 inches of white American women.

Indian mothers at 7th month of pregnancy were 20% underweight as compared with the pregravid weight of 56 kg. for American white women. Out of 200 mothers, 117 were below 44.5 kg. 41 below 41 kg. and 18 below 31 kg.

The weight gain during the last 2 months of pregnancy varied from 0.5 to 10 lbs with a mean of 2.06 lbs (69 normal pregnancy), as compared with the mean weight gain of 23 to 25 lbs for white women. Six women lost weight from 0.5 to 1.5 lbs ; 4 did not show any weight gain.

CLINICAL DATA

The general health of majority of the pregnant women were far from satisfactory. There were 24 women who were grossly undernourished, thin built, emaciated and complained of weakness. Many of them did not show weight gain, some of them lost weight during last 2 months.

Oral hygiene was very poor. Dental caries involving from 2 to more than 5 teeth were seen in 82 cases. In 2 cases all the teeth had fallen out.

Vitamin Deficiency.—Symptoms of vitamin B deficiency was present in severe form in 16 cases, and in mild form in 62 cases. There was a case of nightblindness and 1 of xerophthalmia.

Anaemia :—Anæmia in mild form was present in 78 cases, the Hb% varying from 8 to 10 g.; and in severe form in 24 cases, the Hb conc. was below 6.5 g.% and R.B.C. below 2.33 million per c. mm. The last group of patients had to be admitted to the hospital for treatment.

Toxæmia.—Toxæmia in severe form developed in 5 cases and in mild form in 14 cases. Oedema was present in 12 cases, albumin in urine in 10 cases. The blood pressure at ninth month varied from 130/90 to 220/100.

The weight gain in 12 of the 19 cases was over 41 lbs per month as compared with 2.06 g. in normal group. All showing abnormal weight gain did not develop toxæmia.

The incidence was more among young women between the age of 17 to 22 years and included 11 primipara. There were 6 premature deliveries in toxic group including 2 twin deliveries. Of the newborn 3 babies died after birth.

Other complications.—In the series there were 5 cases of tuberculosis and 3 cases of osteomalacia, both confirmed by X-ray. In addition, 5 had syphilis, 1 diabetes and 1 had heart disease.

The results of clinical study reveals that out of 200 cases only 54 cases may be considered as clinically normal.

BIOCHEMICAL ASSESSMENT

Haemoglobin.—There were only 16 cases with Hb% above 12 g., and 20 cases with Hb varying between 10 to 12 g%; values which are regarded as the lower limit compatible with health during pregnancy and values below 10 g.% regarded as diagnostic of anæmia. The value of Hb varying from 6.5 to 12.4 g% were significantly lower than the values of 11 to 14 g% reported by Paul for the poor and middle class pregnant women in North India.

Supplement of iron in 16 cases produced a rise of Hb from 8.8 g. at 7th month to 10.47 g.% one month postnatal.

SERUM PROTEIN AND SERUM CALCIUM

The mean value of total protein in 16 cases of normal pregnancy was 6.73 g.%, agrees with the mean of 6.14 for white women. The mean values of albumin for the normal group was 3.7 g%; for the 36 patients of anæmia the mean decreased to 3.02 g% and showed a marked decrease to 2.1 g% in 3 cases of toxæmia. The values of serum protein were within lower range or normal in most of the cases.

The values of serum proteins are not dependable criteria of nutritional status of all individuals, values of serum proteins were within normal limits even though clinical malnutrition was present in some cases. The same remark applies to serum calcium. The values of serum calcium varied from 8.25 to 10.5 mg.% with a drop to the mean of 7.4 mg% in 11 cases of toxæmia.

There was, however, a significant correlation between the level of Hb and the clinical condition.

STUDIES OF NEWBORN

Of the 200 cases studied, only 102 delivered in the hospital. Of these, 45 were males and 57 females. Only 79 were considered as full term normal infants. Of the full term infants 10 died from periods varying from 24 hours to 1 month after birth.

Height and Weight.—The mean birth weight was 5 lb-13 oz. and birth length 17·9 inches. These figures are regarded as upper limit of immaturity according to workers in Europe and U.S.A. The reported birth weight for white infants varies from 6 lb-13 oz. to 7 lb-4 oz.

There are indications that birth weight of infants born of higher income group is likely to be higher. The birth weight of infants was found to be 6 lb-2 oz. (Suktankar ; Ramcharan and Roy). In 3 cases in present series from well-to-do families the birth weight varied from 5-13 to 8 lb-4 oz. with a mean of 7 lb-3 oz. agrees with the mean of white infants.

MATERNAL AND INFANT RELATIONSHIP (Hb%)

The Hb% of the cord blood varied from 13·5 to 19g.% (33 cases) with a mean of 16·51 g% as compared with a mean of 19·5 g% for white infant.

The Hb% of the cord blood remained within normal limits (from 15·3 to 17·5 g%) inspite of mothers Hb showing a wide variation from 6·93 to 13·1 g% (33 cases). Infants born of anæmic mothers show a greater fall in Hb% as compared with those born of normal mothers, during period of growth.

*Increase in weight during 8 months :—*The average weight gain during 8 months (33) was 4·47 oz. as compared with usual weight gain in infants of 6 oz. per week up to the age of 6 months.

DIETARY INTAKE

Satisfactory record of food intake could be obtained in 75 cases consisting of 11 vegetarian and 64 women used to mixed diet.

The diet was adequate with respect to calorie; intake in 23 cases providing from 2100 to 2500 calories; but grossly deficient in 20 others in whom the intake varied from 1600 to 1700 calories.

Carbohydrates provided 70 to 80% of the calories. Fat was taken mostly in the form of vegetable oils and dalda, varying in quantities from less than an ounce in 26 cases to 2 to 3 ounces in only 15 women.

The protein intake was less than 50 gg. per day in all the vegetarian group and also in 17 others used to mixed diet. In the whole series, protein of high biological value either from milk or animal sources varied from 5 to 20 g. per day in 90% of the cases.

Calcium intake was less than half the recommended allowance of 1·5 g. per day, the diet being unbalanced with respect to Ca and P.

The diet of the pregnant women was grossly deficient in most of the vitamins, particularly in vitamin A and C. The recommended allowance of 50 mg. of vitamin C was present in the diet of only 10 pregnant women. In the absence of animal fat, green vegetables formed the only source of provitamin A.

The results of dietary and clinical studies agree in indicating that malnutrition resulting from inferior or marginal dietary of long duration poor prenatal care have reflected in restricted and stunted growth, impaired nutritional stability and subnormal health both of the mothers and of their newborn infants. The effect of such marked deprivation in one epoch of life may be carried over to the succeeding period of life cycle and thus affecting the growth, development and the function of mothers and of the successive generation to follow.

5. Inquiry on the estimation of 17-ketosteroids content of urine in toxæmia in pregnancy with special reference to aetiology and prognosis under Dr. S. Mitra, Chittaranjan Cancer Hospital, Calcutta.

One hundred and fifty-six cases of normal pregnancy against 93 cases of toxæmic pregnancy (total 249 cases) have been studied.

The chemical assay method as suggested by the British Medical Research Committee on clinical endocrinology (1952) has been followed.

One is tempted to associate the adrenal cortical activity with the aetiology of toxæmia and it was the purpose of this inquiry to find out any correlation between the adrenal cortex and toxæmia with urinary 17-ketosteroids as the index.

As previously reported, we could not come to any significant conclusion to accept Seyle's suggestion that toxæmia of pregnancy was a 'disease of adaptation'. That report was based on 122 normal and 50 toxæmic cases. From the present series of 249 cases (156 normal and 93 toxæmic cases) our previous conclusion is substantiated. No significant variation in the excretion of adrenal cortical hormone in toxæmia from normal pregnancy has been found.

Taking Tampan's figure of a daily output of 6.4784 mg. as the mean output in normal non-pregnant Indian women, it has been found that there is a definite increase in level in both normal and toxæmic pregnancies. The average daily output in toxæmia has been found to be 10.88 ± 0.62 mg. per 24 hours (Tampan 10.07 mg.) and in normal pregnancy 10.81 ± 0.65 mg. per 24 hours (Tampan 9.52 mg.) No significant difference in values between normal and toxæmic pregnancies has been observed in our series.

6. Inquiry on Rh factor in pregnant mothers and its importance in toxæmia of pregnancy and hæmolytic disease of the newborn under Dr. Y.G. Bhide at the J.J. Groups of Hospitals, Bombay.

Out of 2,020 women, 37 showed albumin in various degrees in urine, while the blood pressure above 140 was found in 13 cases. None

of these include the 20 patients who were Rh-negative. The blood pressure above 140 and also the presence of albumin in urine, have been taken as the indicators of toxæmia of pregnancy. As will be seen, the series of Rh negative patients do not show either of these, it may be considered that Rh-factor has no major role in toxæmias pregnancy. Further the Coombs' test which is a standard one employed in the diagnosis of cases of erythroblastosis fœtalis, has not been found to be positive in any of 20 cases. Out of these 20 cases, 12 were in *primi para*. With a view to rule out ABO sensitization, the ABO antibody titres were determined in 570 cases but none showed high titres. Out of 2,020 pregnant women, 20 were Rh-negative, while 2,000 were Rh-positive. The Rh-negative percentage comes to one per cent.

7. Study in Rh sensitization under Dr. V.S. Mangalik at the K.G. Medical College, Lucknow.

1. *Procedure of work*.—Expectant mothers, attending the antenatal clinic of Queen Mary's Hospital, K.G. Medical College, Lucknow, and Government Dufferin Hospital, Lucknow, formed the subjects of this study. A record was entered of any abortion, premature delivery, or still-birth in the past or present pregnancy. In cases who delivered in the hospital, the health of the newborn child at birth and in the immediate postnatal period was followed. The records of the women investigated are taken on the same lines as recommended in the Indian Council of Medical Research schedule.

ABO and Rh (D) blood groups were determined in these women. D-negative blood samples were further tested for the presence of C, c, E, and e antigens. These samples were also studied for the detection of Du antigen by the use of anti-human globulin serum. It has not been possible to carry out a complete genotyping in all the Rh-negative women because of the delay in the availability of the immune sera. All sera obtained from Rh-negative mothers, whether primipara or multipara, and whether they gave a history of repeated abortions or not, were examined for the presence of antibodies against D, both in saline and protein menstrum. Attempts were made to contact the husbands and children of these Rh-negative mothers for the determination of Rh (D) and ABO blood groups. It has not, however, been possible to do so in many cases. Either the family members live outside the town, or if they can be contacted in the town, they refuse to give blood.

2. *Techniques employed*.—All the immune sera employed was manufactured by Ortho-Pharmaceutical Corporation, New Jersey U.S.A. They were of uniform high titre and avidity.

Samples of blood were obtained from the vein, and all tests were carried out on clotted blood.

(a) *Detection of Rh (D)*.—Slide technique for the detection of D antigen was employed. A drop of anti-D slide test serum was put on

a glass-slide previously warmed (surface temperature 40°C.-45°C). Two stick-full suspension of the cells from the clot of the blood to be tested were mixed with the serum (forming roughly a 2 per cent suspension) with the applicator. Agglutination was looked for after two minutes. All negative results were checked microscopically.

(b) *Detection of Du.*—A red cell suspension (2 per cent) was prepared in normal saline from the clotted blood. To two drops of this suspension in a small test-tube, was added one drop of anti-D slide test serum. The mixture was incubated at 37°C. for one hour. After this period the cells were thoroughly washed three times, with normal saline and decanted completely after the last washing. The drops of anti-human globulin serum were then added, the mixture shaken well and centrifuged at 800 r.p.m. for 1 minute. Agglutination was looked for by gentle agitation and also microscopically.

(c) *Detection of anti-bodies in Rh-negative women.*—The following procedures were adopted in routine for the detection of antibodies in each case :—

- (i) Two drops of the serum to be tested was mixed with two drops of a 2 per cent suspension of Rh positive, O-group cells in normal saline in a test-tube. The mixture was incubated at 37°C. for 1 hour and examined for agglutination.
- (ii) The above procedure was repeated using a 2 per cent red cell suspension in 20 per cent bovine albumin solution.
- (iii) *Direct Coomb's test.*—Two drops of a 2 per cent normal saline suspension of cells were washed three times with normal saline. After the last washing, the cells were completely decanted off and two drops of anti-human globulin serum were added to them. The mixture was mixed well, centrifuged at 800 r.p.m. for 1 minute and read for agglutination both by naked eye and microscope.
- (iv) *Indirect Coomb's test.*—A mixture of two drops of 2 per cent saline suspension of Rh positive, O-group cells and two drops of the serum to be tested was incubated at 37°C. for 1 hour. Next, the cells were washed three times with normal saline, and two drops of anti-human globulin serum added. The mixture was well shaken, centrifuged at 800 r.p.m. for 1 minute, and read for agglutination.

RESULTS

Incidence of Rh factor in expectant women.

Total tested	Rh (D) positive	Rh negative	Rh negative per cent.
1,444	1,435	9	0.62

Distribution of ABO groups in Rh-negative women.

Total	Group 'A'	Per cent	Group 'B'	Per cent	Group 'AB'	Per cent	Group 'O'	Per cent
9	4	44·5	1	11·1	2	22·2	2	22·2

Distribution of ABO groups in Rh positive women.

Total	Group 'A'	Per cent	Group 'B'	Per cent	Group 'AB'	Per cent	Group 'O'	Per cent
1431,	297	20·7	573	40	180	12·6	381	26·7

Incidence of abortions in Rh-negative and Rh-positive women.

Group	Total cases	Number of abortions			Per cent of total cases
		1 to 2	more than 2	total	
Rh-positive	1,435	381	16	537	37·4
Rh-negative	9	4	55	9	100

Comments.—1,444 specimens of blood obtained from pregnant women attending the antenatal clinic have been tested for ABO and Rh (D) blood groups. Of these only 9 women have been found to be D-negative (0·62 per cent.) The percentage of women who gave a history of abortion is significantly higher in the Rh-negative group. Women giving a history of repeated abortions are twice as many in the Rh-negative group as in the Rh-positive group. Anti-D-antibodies have not, however, been demonstrated in any case so far.

The incidence of group B is the highest in these women as compared with A, AB and O groups. In the Rh-negative group the reverse holds good. The data are, however, too meagre to warrant a valid conclusion.

8. Study of Rh factor in pregnant mothers in Indian States and its importance, if any, in toxæmias, still-births, neonatal deaths and haemorrhagic diseases of the new-born under the Principal, Christian Medical College, Ludhiana.

During the past year the work has continued and 571 groupings have been carried out as follows :—

Pregnant women from antenatal clinics and wards	...	500
Non-pregnant women from general wards	...	46
Men		25
TOTAL		571

Antenatal cases first had a general and obstetric history given and the blood was examined for ABO group, Rh (D,d antigens) and Kahn test. Cases found to be Rh negative (d) were subject to antibody tests.

SUMMARY OF FINDINGS. (*including the 100 cases grouped last year*).

Incidence of groups according to caste or community.

ABO group Community :	O	B	A	AB	Rh positive only (ABO not)
Hindu (437)	137 (31·5 per cent)	179 (41·3 per cent)	93 (21·4 per cent)	25 (5·8 per cent)	3
Sikh (215)	73 (34·8 per cent)	81 (38·6 per cent)	44 (20·9 per cent)	12 (5·7 per cent)	5
Christian (18)	5	9	...	4	
Muslims (1)		1			
Totals	215 (32·43 per cent)	270 (40·72 per cent)	137 (20·66 per cent)	41 (6·19 per cent)	

Incidence of Rh group

Rh negative (dd)	Hindus (30 out of 437)	...	6·84 per cent.
	Sikhs (19 out of 215)	...	8·84 per cent.
	Overall (51 out of 671)	...	7·66 per cent.

Among the 468 women found to be Rh positive, 282 (60·6 per cent.) gave some kind of abnormal obstetric history (at least one abortion) and of these 126 (26·9 per cent) gave a history of still-births or neonatal deaths.

Among the 32 Rh negative women, grouped this year, 22 (68·75 per cent) gave an abnormal obstetric history of some kind.

9(28·1 per cent) gave a history of neonatal deaths or stillbirths.

No Rh antibodies were detected and the reasons for this are discussed, with particular reference to the potency of Coomb's reagent. Two women were suspected to be cases of Rh immunization, one of whom had not been pregnant for two and a-half-years. These cases, and three others are given in detail, with the relevant laboratory findings.

Syphilis (as shown by a positive Kahn test) was found in 6·0 per cent of the Rh positive women and 6·25 per cent of the Rh negative women ; it was not a serious complication in assessing the role of the Rh factor in causing maternal and neonatal ill-health.

No relationship was discovered between the incidence of the Rh negative (d) antigen and that of toxæmia of pregnancy and haemorrhagic disease of the newborn.

9. Studies on physical developments of babies and dietetic consumption of mothers in pre-natal periods under Dr. (Smt.) P.K. Verma, Medical Superintendent, Rafat Maternity Home, Rampur, U.P.

The object of the enquiry was to find out the effect of different food-stuffs in daily diet of mothers in antenatal periods on the birth weight of the babies.

For this inquiry the mothers were contacted in the pre-natal period, in the antenatal clinics. On the first contact with the mother a clinical assessment of the physical nutritional condition was made and the mothers who were in average state of health were taken for further follow up. These mothers were asked to keep a daily diary of the dietetic intake. If the mothers kept up normal throughout the pregnancy and did not develop any abnormality which could have any bearing on the mothers general health—the baby was included for study. Only full time normal babies were listed.

Altogether 500 mothers were investigated and their babies weighed at birth. The effect of the consumption of the following food-stuffs of diet, in the prenatal period, on the birth weight of the babies were investigated :—

1. Meat, fish—eggs etc.
2. Cereals.
3. Milk and milk products.
4. Vegetables.
5. Pulses and Dals.
6. Fats.
7. Sugars.

1. MEAT, FISH, EGGS ETC.—

Out of the 500 mothers whose diet was investigated 131 was vegetarians and 369 were meat eaters. It was observed that the average birth weight of the babies of the mothers taking vegetarian diet was 6 lbs.—2 ozs. as compared to the babies of mothers taking a mixed diet, whose average birth weight was 6 lbs. & 9 ozs. Further it was noticed that nearly 42 per cent of the babies of the mixed diet group had their birth weight above this mean average birth weight while only 17 per cent had their birth weight below 6 lbs. While in the case of vegetarian mothers 31 per cent of the babies had birth weight below 6 lbs. and only 27·6 per cent above the average.

2. CEREALS

The cereals used as the main diet in this group were rice and wheat. The average daily consumption of each mother varying between 10 ozs.—12 ozs. per day in 95 per cent. Only 92 mothers were principally rice eaters, the remaining 408 were taking wheat as their staple diet. It was seen that the average birth weight in the babies of rice eating mothers was 4 lbs. and 8 ozs. only whereas in the group taking wheat as the principal diet the average weight of the baby at birth was 6 lbs. and 8½ ozs. The same was further observed in vegetarian and non-vegetarian group separately.

Average weight of babies of rice-eaters vegetarian mothers was	...	4 lbs. and 4½ ozs.
Average birth weight of babies of non-vegetarian rice eaters	...	4 lbs. and 9½ ozs.

3. DALS—PULSES

The mothers investigated in the group showed that the diet of non-vegetarian mothers had very little of the Dals etc. The average consumption being only ½ oz. to 1 oz. Dals daily. The vegetarian mothers were consuming more of Dals and pulses the least consumption being 12 ozs. in a week. It was observed that with the increase of pulses in the diet there was a rise in the birth weight of the baby.

4. MILK AND MILK PRODUCTS

The intake of the milk and its products was very low in this group. 279 mothers were taking milk in tea 1—1½ ozs. only daily or none at all. 221 mothers were taking 8 ozs. or more of milk daily in the diet. It was observed that the average birth weight of the babies of these mothers was 6 lbs.—11 ozs. while in the group not consuming any milk it was only 6 lbs. ½ oz. The same effect was observed in vegetarian and non-vegetarian groups separately.

Vegetarian with milk—Average birth weight was— 6 lbs. & 12¾ ozs.

-do-	without milk—	-do-	5 lbs. & 7¼ ozs.
Non-vegetarians	with milk—	-do-	6 lbs. & 11 ozs.
-do-	without milk—	-do-	6 lbs. & 7 ozs.

5. VEGETABLES

Average daily intake of the vegetables in the group under investigation was very low being only between 2 ozs. to 8 ozs. daily including roots and tubers, green leafy vegetables and other type of vegetables. No effect of the same could be judged on the birth-weight of the babies.

6. FATS

It was observed in this group that most of the mothers were consuming only vegetable fats. *i. e.* mustard oil and vegetable ghee-

Only 5 per cent. of the mothers were using any ghee or butter. The daily consumption was $\frac{1}{2}$ to 1 oz. which being the same nearly in all the mothers under observation. No effect on the birth weight was appreciable.

7. SUGARS

The mothers under observation were taking on an average 1 oz. of sugar or gur daily, but the consumption has no noticeable effect on the birth weight of the babies.

CONCLUSIONS DRAWN

(a) The animal nitrogenous food stuffs like meat, fish etc. in the mothers diet during the pre-natal periods tends to raise the birth-weight of the babies.

(b) Wheat as a staple cereal diet in the pre-natal periods has advantage over the rice in raising the birth weight of the babies.

(c) Milk and milk products in a diet of the mother tends to raise the weight of the babies.

(d) Pulses and Dals consumed in the pre-natal periods have some effect on the rise of the birth weight specially in the mothers who do not take any meat etc.

(e) Vegetables and fruits have no special effect on the birth weight of the babies.

(f) Fats and sugar do not have any noticeable effect on the birth weight of the baby.

10. Inquiry into the relationship between deficiency of vitamin B complex and toxæmias of pregnancy under Drs. M. L. Pai and Thakor V. Patel at the Medical College, Baroda.

Synopsis of the work and results achieved.—Toxæmias of pregnancy forms a major problem to obstetricians, not only in India but in other countries as well. Maternal as well as foetal mortality, resulting from it, is a very common phenomenon. Thus, as a cause of maternal and foetal mortality, it ranks next to anæmias in this country. When mineral and vitamin intakes are low and mineral metabolism defective during pregnancy, not only are the results predisposition to bone and tooth defects in the foetus but also the associated nutritional disturbances in the mother may be one of the etiological factors in vomiting, eclampsia, etc. (Macy and Mack, *Amer. Jour. Obstet. & Gynæc.*, **68**, p. 131, 1954)

In the preliminary work, urinary nicotinic-acid excretion levels in twenty normal healthy female subjects were estimated (Pai, *Jour. M.S. Univ. of Baroda*, **4**, p. 49, 1955). Later, in the continuation of the preliminary work, fifteen cases of toxæmias of pregnancy were studied for their urinary nicotinic acid excretion levels (Pai, *Jour. Ani. Morph. & Hum. Physiol.*, 1956, in press *vide Graph*). The results of the study of the preliminary data have been represented in the Graphs.

In order that there should be comparative data available for assessing the state of deficiency of the vitamins, namely thiamin, riboflavin and nicotinic acid, in cases of toxæmias of pregnancy, study in normal healthy subjects was necessary. Such subjects were selected from the Nursing Staff of the S. S. G. Hospital, Baroda. They are being studied for their urinary excretion levels of these vitamins, both before the saturation test dose and after the administration of the latter in order to assess the state of saturation of their body tissues in respect of these vitamins. Their dietary intake has been recorded. These estimations have been done during the particular period of interval of menstrual cycle, i.e., between the 10th and 18th day of the cycle. Some more data on the normal subjects are needed, which are, therefore, being studied.

A very close relationship exists between a deficiency of vitamin B₃ complex and the occurrence of toxæmia of pregnancy. In the present series, the urinary excretion of nicotinic acid in 24 hours collection has been found to vary between 1.01 mg. and 3.0 mg. with an average of 2.24 ± 0.17 mg./day. This range can be compared with that obtained in normal healthy individuals (*vide* Graph). In case of the normal healthy subjects the excretion of nicotinic acid in 24 hours sample of urine was found to vary between 4.6 mg. and 7.9 mg. with an average of 5.56 ± 0.21 mg./day. Thus, the urinary excretion of this vitamin in the present cases of toxæmias of pregnancy is lower when compared with the normal urinary excretion and it is about slightly less than 50 per cent of the latter. This lower urinary excretion of the vitamin in the cases of toxæmia of pregnancy indicates that there possibly exists a close relationship between the deficiency of this vitamin, namely nicotinic acid and the occurrence of toxæmias of pregnancy. Of course, it must be pointed out here that the criterion taken in this study for judging the deficiency of the vitamin in the patients is the 24-hour urinary excretion of the latter. Though this criterion will, to a large extent, be serving as one of the indications towards judging the deficiency of the vitamin concerned, still, however, other tests, such as the saturation tests when carried out as supplementary tests to this single urinary estimation, will no doubt be giving an added evidence in arriving at a definite conclusion.

It is intended to extend this work further to the determination of the excretion of other two vitamins of the B complex group, i.e. thiamine and riboflavin (besides the nicotinic acid), after adopting the saturation test-dose technique.

11. Inquiry into the causes of prenatal death under Dr. A.C. Bhowmick and Dr. S. De at the Medical College, Calcutta.

This inquiry was started on 21st June, 1956 with grant-in-aid from the Indian Council of Medical Research. The work is being done in the Department of Obstetrics and Gynæcology, Medical College, Calcutta. The term 'prenatal death' is used to mean the death of the foetus before birth and of the new born in the first 10 days of life. The object of the work is determination of the causes of prenatal deaths by post-mortem examination of selected tissues.

Up to the end of September 1956, it has been possible to make 48 post-mortem examinations. Of these, 25 were on still-born fœtuses and 23 were on live-born babies that died within the 1st 10 days of life. It is premature to draw any conclusion on this small number of cases. So far, the autopsy finding revealed the following causes of still-births and neonatal deaths:—

A. Causes of still-birth :

(i)	Trauma—including intracranial hæmorrhage, intracranial stress and other injuries	...	...	8
(ii)	Intra-uterine asphyxia	...	...	8
(iii)	Developmental defects	...	...	3
(iv)	Hæmolytic diseases	...	...	1
(v)	Anæmic Anoxia	...	...	1
(vi)	Undetermined	...	...	4
TOTAL				25

B. Causes of neonatal deaths :

(i)	Trauma—including Intracranial hæmorrhage, and Intracranial stress	...	...	5
(ii)	Asphyxia	...	...	3
(iii)	Atelectasis	...	...	5
(iv)	Bronchopneumonia	...	...	2
(v)	Hæmolytic disease	...	...	2
(vi)	Hæmorrhagic disease	...	...	2
(vii)	Developmental defect	...	...	1
(viii)	Gross Prematurity	...	...	3
TOTAL				23

Out of the 23 neonatal deaths, 18 were premature and only 5 mature, whereas among the 25 still-born babies only 12 were premature and 13 were mature.

Examination of the physical characteristics of the heated oil showed a significant reduction in iodine number. Digestibility of these oils by lipase was also reduced.

Vitamin B₁₂ did not afford any protection against the adverse symptoms produced by the heated oil. On the other hand, liver extract nearly completely eliminated the fat accumulation in the liver, though it had no effect in overcoming the growth inhibition.

It was found that a high-protein diet (25 per cent cæsin) proved of immense benefit to the animals in as much as both the retardation in growth and accumulation of fat in the liver were eliminated. Vitamin B₁₂ in combination with a high-protein diet was found to be somewhat beneficial.

Mustard oil, on heating to the same temperature and time, did not produce the adverse effects observed with the other three oils. This aspect is being studied.

Experiments are also in progress to find out the effects of feeding heated oils to rats on a long-term basis.

12. Study of nutritional status of expectant mothers with particular reference to the incidence of toxæmias of pregnancy and anæmias in pregnancy under Dr. V.S. Mangalik at the K.G. Medical College, Lucknow.

Procedure of work.—Two types of cases were included in the study. In the first group, women between 20 and 28 weeks of pregnancy were selected, while in the second group, cases of toxæmias of pregnancy and anæmias in pregnancy who were above 28 weeks of pregnancy, were included. At the first visit of the patient, a detailed history was taken which included a past and present obstetric history and dietetic intake. A complete physical examination was carried out including urine examination for the presence of albumin, record of blood pressure and hæmatologic and biochemical investigations. On subsequent monthly visits, clinical examination, Hb. estimation, urine examination and blood pressure and weight records were repeated. At term biochemical and hæmatologic examinations were again undertaken. Any abnormality in labour was noted and cord blood collected for the estimation of Hb. content of foetal blood and if that was not available, blood from the baby was obtained directly within 24 hours of birth. Weight and length record of the baby was also kept.

CONCLUSIONS

Cases studied.—Total number of cases studied from May 1, 1956 to September 30, 1956, have been 159. Out of these, 130 were those which had been registered in the antenatal clinic before 28 weeks of pregnancy and the rest 29 cases were those which had either never attended the antenatal clinic and were admitted directly into the hospital or were above 28 weeks of pregnancy. Out of the first group of cases, two cases showed signs of toxæmia and 72 cases were found to be anæmic. In the second group, 9 cases were of toxæmia and 17 cases of anæmia and 2 normal cases above 28 weeks of pregnancy.

Social status.—Majority of the patients in both the groups belonged to poor and lower middle class families. Out of the total recorded cases, 63 per cent had a family income of less than Rs. 100/- per month.

Nutritional status.—The quantity and the type of diet in the majority of cases was very poor. Most of the patients were taking a mixed diet which comprised mainly of wheat flour, rice, dal, cheaper variety of vegetable with occasional addition of meat or fish. Milk, fruits and eggs were seldom included in the diet.

Clinical findings.—Most of the patients in both the groups were underweight and undersized. They often showed signs of deficiency in the form of oral and ocular manifestation. It was observed that the average serum proteins were 6.29 g. per cent and that a definite fall occurred as pregnancy reached term. In the pre-eclamptic and eclamptic patients, the fall in the serum proteins was even greater with a definite lowering of the albumin fraction. No significant variation in the serum calcium and phosphorous levels was found as pregnancy reached term.

We have not observed any significant difference in the distribution of ABO blood groups in toxæmic patients as compared with the non-toxæmic groups of mothers. We have not observed any Rh incompatibility in toxæmia of pregnancy. The salient biochemical and hæmatologic findings are given in the Table :—

Eclampsia (total cases 4)

Serum protiens		Serum calcium		Serum phosphorus		P.C.V.		Hb		Blood groups			
Normal	+	Normal	+	Normal	+	Normal	+	Normal	+	A	B	AB	O
1	...	2	1	4	0	...	3	1	2	...	2	...	2

Pre-eclampsia (total cases 7)

3	2	2	3	1	3	6	...	1	5	...	2	3	3	1	0
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Anæmia (total cases 89)

55	12	12	68	4	17	77	3	9	65	0	24	...	...	89	29	35	13	12
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13. Study of nutritional status of expectant mothers with particular reference to the incidence of toxæmias of pregnancy and anæmias in pregnancy under Dr. P.K. Devi at the Medical College, Nagpur.

It was decided, at the outset, to investigate this problem under two heads :—

(a) Nutritional assessment of 200 pregnant mothers attending the antenatal clinic and centre at the Medical College, Nagpur.

(b) Nutritional assessment of all anæmias and toxæmias of pregnancy admitted in the Professor's unit at the Medical College Hospital, Nagpur.

The following criteria were used to select cases : (1) The patient should be a permanent resident of Nagpur and should be able to give her correct address, (2) she should attend the hospital for delivery except in the cases selected from the MCH centre where the hospital staff would attend the confinement. The MCH Centre cases were included because liberal UNICEF dietary supplements were supplied to them and it would be interesting to note the difference between Hospital cases and MCH centre cases.

So far, the schedule has been completed for 100 cases from the antenatal clinic out of which eighteen cases have delivered. In group (b), only eight cases have been studied so far. The following brief analysis of 100 cases in group (a) is given below :—

Economic and social status.—From the total earnings and the total number of family members the per capita income was calculated. It is surprising how many women are completely ignorant of their husband's earnings or income. The per capita income was less than Rs. 15 per month in 8 per cent of cases, between Rs. 15 and 30 in 45 per cent between Rs. 30 and 50 in 26 per cent and above Rs. 50 in 8 per cent of cases. The first group mostly constituted of daily wage labourers, the second group were mill workers, the third group from lower paid government servants and shopkeepers and the fourth group from upper class of government servants.

SIGNS OF DEFICIENCY

Xerosis—68 per cent.

Vascularization of the cornea—3 per cent.

Glossitis—30 per cent.

Angular stomatitis—22 per cent.

Gingivitis—59 per cent.

Chalky teeth—13 per cent.

Caries—3 per cent.

Edema—18 per cent.

Anorexia—41 per cent.

Diarrhœa—7 per cent.

Liver enlargement—1 per cent.

Thus, it will be seen that the most common manifestations are xerosis and gingivitis.

Hæmoglobin estimations were done on all cases by Sahli's method.

Less than 6 g. per cent.—6 cases (minimum was 2·5 g.).

Between 6·1 and 8 g.—41 cases

„ 8·1 and 10g.—50 cases

Over 10 g.—3 cases

Serum proteins were generally low. Ninety per cent of the cases had total serum protein levels between 4·5 and 5 g. per cent 5 per cent above 5 g. and 5 per cent below 4·5 g.

There was no change in the albumin globulin ratio.

Diet.—Sixty-nine cases were exclusive vegetarians, 7 cases taking more than 20 oz. of milk daily. Out of the 31 non-vegetarians, 25 belonged to the 'occasional' group taking meat or fish, not oftener than once a week. Considering all aspects, 13 cases were thought to have a diet adequate as regards proteins and calories.

Deliveries.—Eighteen out of the hundred cases have delivered at the time of presenting this report. There was one maternal death in a case of severe anæmia (2·5 g.) who had a premature still-birth and expired six hours later. The number of cases delivered is too small to make any remarks regarding average birth weight, hæmoglobin, etc. In this group only 8 cases have been studied so far. It has, however, being noticed that the anæmias in group (b) are severe as only patients with less than 6 g. are admitted as in-patients because of shortage of beds. It is, therefore, proposed to investigate these cases in a little more detail than given in the schedule, particularly as regards hæmograms and marrow smears. It is in this group that follow-up studies are likely to be most valuable. The schedule may also be altered to include investigations of serum-iron levels and total iron-binding capacities in view of the almost universal prevalence of iron deficiency anæmias of pregnancy.

14. Study of Rh factor in pregnant mothers under Dr. R.K.K. Tampan at the Government Medical College, attached Hospital, Trivandrum.

Blood of 47 pregnant mothers were studied including the blood of the father and the child.

Of the 47 pregnant women whose blood was studied, we found 6 of them to be Rh negative. Among the children born to these 47 mothers, 7 were Rh negative.

Among the 6 Rh negative mothers who gave birth to children, both the mother and child were Rh negative in 3 cases. In the other three, though the mother was Rh negative, the children were Rh positive.

None of the Rh negative children showed any symptoms of Jaundice, Erythroblastosis or to her manifestations of blood diseases.

Of these 47 pregnant women, the blood of the father could be studied only in 23 and of the latter only one was Rh negative,

where we found the mother also Rh negative and the child Rh positive. Where the 4 children were Rh negative in this group of 23, both father and mother were positive in 2 cases and in the other 2, they were opposite. Studying the distribution of Rh negative children and the mothers ABO blood group, we found that the 7 children were distributed as follows :—

Blood Group	O.	4 Cases.
	A.	1 Case.
	B.	2 Cases.

15. Study of Rh factor in pregnant mothers under Dr. P.K. Devi at the Medical College, Nagpur.

Six cases from the clinic were selected for study ; the only condition being that the patient should attend the hospital for confinement and a sample of husband's blood would be available.

Whole blood was used in all cases. The following points are particularly worth mentioning. Tube tests were carried out for all Rh determinations. maternal antibodies were tested for, in both saline and albumin media and indirect Coomb's test carried out in all cases. direct Coomb's test was carried out on all cord blood samples in addition to ABO-grouping and Rh-grouping and where antibodies were detected in the maternal blood titre was determined. ORTHO diagnostic reagents were used in all cases.

A total of 283 Rh typings were done, 173 in women, 58 in men, and 52 on cord blood. Of the 173 antenatal cases examined, 170 cases belonged to the first group and three cases to the second group. Schedules were completed for all cases. There were five women who were Rh-negative in the first group out of 170 cases. Of these, three patients showed no antibodies, two of them being primigravida and the third a second gravida. The third case mentioned above has since delivered in hospital, the cord blood showing a negative direct Coombs' test. Two of the five Rh negative cases showed anti-bodies even at the first examination, one showing only the saline type, the other showing both saline and albumin types. The obstetric history in these two cases is interesting in that the first case has three living children followed by two abortions and the second case has had two premature deliveries with two neonatal deaths. In both cases there is no history of previous blood transfusion. The details of all these cases will be submitted in the final report.

Of the 58 male blood examined, all were Rh positive, so also all 52 cord bloods. Of the 170 cases in the schedule, 52 cases have delivered up to the time of this report. In these cases there were 33 cases of ABO-group incompatibility and one case of Rh incompatibility between the maternal and foetal bloods but the direct Coombs' test on all cord bloods was negative as reported above.

Of the three cases in the second group, one was a case of hemolytic anæmia in the new-born referred for investigation, where it was found that the mother who was Rh negative had presumably been sensitized by a previous transfusion. The other two were Rh positive but showed only weak agglutination.

16. Study of Rh factor in pregnant mothers under Dr. Subodh Mitra and Dr. Santosh Mitra in collaboration with Dr. C.R. Das Gupta at the Chittaranjan Cancer Hospital, Calcutta.

The period of work covered four and a-half months, from May 16, 1956 to September 30, 1956, both inclusive. The materials of the study were collected from the Obstetric Department of Chittaranjan Seva Sadan College of Obstetrics, Gynæcology & Child Health and its hospitals. The technique recommended in the schedule by ICMR was being followed for Coomb's test and the modified technique suggested by Dr. C.R. Das Gupta was followed for Rh-factor determination. The modified technique is as follows :—

With separate capillary pipettes equal amounts of washed red blood cells in normal saline and clear serum of the patient are put side by side on a clean glass-slide. With another capillary pipette, a small amount of Rh-anti serum is put by the side of the washed red blood cells. All the three were mixed well with a glass-rod and allowed to stand in a moist chamber. Clearcut results visible to the naked eye, are obtained in all the cases within a few minutes. The results obtained with the two techniques, the one described in the schedule and the modified technique by Dr. C.R. Das Gupta were compared in a number of cases and were found to be identical in all the cases. As the amount of anti-serum for the modified technique is much less, the modified technique is being routinely used to determine the Rh of the patients. This has resulted in the saving of considerable amount of anti-serum and hence in expense as well.

One hundred forty-seven pregnant mothers, 39 husbands and 16 cord blood samples were studied. Among these six cases which include one cord blood proved to be Rh negative. Out of these six negative cases one gave Coombs test weekly positive at her 27th week of gestation, she was 18 years of age, primigravida without any history of blood transfusion or intramuscular injection.

Blood grouping was done in 169 cases of which 46 were of group A, 65 group B, 7 group AB and 51 were of group O.

As there was lack of co-operation on the part of the pregnant women and their husbands, the detailed study particularly in the time of follow-up was not possible, however, a blue card system has been introduced in the antenatal department of the said institution with assurance of admission to the hospital if the pregnant mothers furnish that card nearabout the expected date of confinement.

The meagre data obtained so far is not sufficient enough to come to any conclusion. It, however, seems that about 3 per cent of the population of the above-named hospital may be Rh negative and majority of them are of B group, next comes O group and then A group and last comes AB group. Further work will be necessary for a statistical significant conclusion.

NUTRITION

1. Nutrition Research Laboratories, Coonoor

I. STUDIES ON VITAMINS

VITAMIN A

The utilization and storage of vitamin A in the body is apparently influenced by several dietary factors but there is little conclusive evidence on the relative roles of the different nutrients as also on the influence of total energy intake. Some of these aspects are under investigation as a part of a comprehensive approach to this problem. Observations on the influence of dietary protein were reported a few years ago (*Ann. Rep.*, 1951-52, p. 2). The rôle of phosphorus content of the diet on vitamin A utilization and storage by the albino rat was recently investigated. The results indicated that the phosphorus content of diet did not influence the absorption of carotene nor its utilization in the albino rat.

VITAMIN B₁₂

Absence of vitamin B₁₂ activity in pulses.—Rohatgi *et al.* (*Jour. Nutr.*, **56**, p. 403, 1955) have reported some vitamin B₁₂ activity in pulses, but as they did not subject the pulse extracts to alkali treatment their results left room for doubt as to the true nature of the microbially active substances reported by them. Hence, vitamin B₁₂ activity was assayed in extracts prepared from the four pulses according to the procedure of Rohatgi *et al.* (*loc. cit.*), before and after alkali treatment, employing *L. leichmannii* (A.T.C.C. 4794) as the test organism. The average values for the untreated extract obtained on three independent estimations of each pulse were as follows: Bengal gram (*Cicer arietinum*) 0.28. Black gram (*Phaseolus mungo*) 0.30. Green gram (*Phaseolus radiatus*) 0.81 and red gram (*Cajanus indicus*) 0.40 g. per 100 g., respectively. Nearly, the same values were obtained after alkali treatment. It seems, therefore, that the factors stimulating the growth of the organism must, in all probability, be substances other than cyanocobalamin.

Vitamin B₁₂ activity of fæces of albino rats maintained on vegetable protein diets.—As there are numerous reports in the literature about the role of vitamin B₁₂ in promoting growth in young, in the better utilization of protein as well as in hæmopoiesis, it was felt necessary to investigate the metabolism of vitamin B₁₂ in rats maintained on vegetable protein diets.

Two-day samples of the fæces were collected from individual rats maintained for four weeks on diets in which protein was derived from a mixture of a cereal, pulse and green leafy vegetables and assayed for B₁₂ activity using *L. leichmannii* as the organism, before and after alkali treatment. The fæces of rats kept on skim milk diets were also analysed for comparison. The fæces of the rice group showed about 54 per cent of the activity found in skim milk group. So far as rats on the other three cereals used in this experiment were concerned, the faecal excretion of vitamin B₁₂ did not differ significantly from that observed on the skim milk diet. Over 98 per cent of the activity was destroyed in each case after alkali treatment which ruled out the possibility of desoxyribosides in the fæces extract.

VITAMIN D

Citrogenase activity of epiphyseal cartilage.—Observations on citrogenase activity in the epiphyseal cartilage of rachitic and vitamin D treated rats using pyruvate as substrate have confirmed the earlier findings made with preformed acetate.

Citrogenase activity in the epiphyseal cartilage of rachitic dogs was lower than in vitamin D treated animals. Preformed citrate in rachitic dog cartilage was also lower than in vitamin D treated animals, thus confirming in a different species of animals the observation made originally in rats that carbohydrate metabolism of the epiphyseal cartilage is adversely affected in vitamin D deficiency. The site of disturbance was also similar in rats and dogs.

The citrate content of rachitic rat epiphyseal cartilage was 2.01 ± 1.26 $\mu\text{g./mg.}$ dry weight. It increased to 3.43 ± 1.40 $\mu\text{g./mg.}$ within 24 hours after vitamin D administration. The level of citrate rose gradually till it was 4.50 ± 1.15 $\mu\text{g./mg.}$ at 144 hours. The citrate content in the epiphyseal cartilage of rats of comparable age on stock diet was found to be 6.44 $\mu\text{g./mg.}$

Total phosphorus in epiphyseal cartilage.—In view of the close relationship between the phosphorus and carbohydrate metabolism, changes in the total P content of rachitic epiphyseal cartilage as affected by treatment with vitamin D were determined. The results showed a progressive increase in total P starting from 9.78 $\mu\text{g./mg.}$ dry cartilage to 20.94 $\mu\text{g./mg.}$ at 96 hours. The peak at 96 hours coincided with the onset of calcification *in vivo*. The fall in total P in the following period still awaits explanation. Total P in rachitic dog epiphyseal cartilage also responded to vitamin D by an increase.

II. STUDIES ON PROTEINS

The global problem of protein malnutrition in infants and children has necessitated a fresh evaluation of available foodstuffs of vegetable origin from the standpoint of the efficiency of their proteins in promoting growth. In the investigations described below, an attempt has been made at such evaluation of cereal and pulse proteins. At the same time, attempts have been directed to test various combinations of foodstuffs in proportions commonly consumed to find out if a dietary protein mixture of high biological value could be evolved. The capacity of such proteins (a) to maintain plasma protein and hæmoglobin levels in blood, and (b) to regenerate them on depletion, is also being tested.

Protein efficiency ratio of vegetable proteins.—(a) Proteins of individual cereals and pluses, (b) mixtures of cereals with pulses, and (c) mixtures of cereals, pulses and green leafy vegetables have been tested for their P. E. ratio in albino rats at 10 per cent level of intake. In cereals containing less than 10 per cent protein, the latter was separated by alkali extraction followed by precipitation and dehydration. The separated protein was then added to the cereal diet to bring its protein content to 10 per cent. In experiments where mixtures were used, the pulse provided 3 per cent protein and leafy vegetable one per cent, the balance of 10 per cent was made up by adjusting the amount of cereals in the diet.

Although, four leafy vegetables were originally selected, only amaranth gave good supplementary value. Hence, amaranth was used in most of the experiments, results of which are described below :—

The following conclusions emerge from these studies :—

1. The P.E. ratio of rice is not improved by either the addition of red gram or amaranth alone or together in the proportions used in these experiments.

2. The P.E. ratio of wheat, bajra and jowar is significantly improved by the addition of the common pluses. The addition of amaranth alone gives variable results. The improvement in P.E. ratio is maintained when pulse and amaranth are added together except in the case of jowar when the pulse component happens to be black or green gram.

3. The P.E. ratio of Bengal gram protein is significantly improved by amaranth, whereas the latter did not exhibit this effect on proteins of the three other pulses.

In general, therefore, it can be concluded that the nutritive value of the protein of the commonly consumed cereal is enhanced by supplementation with pulse and amaranth, rice being the only exception. The reason for the latter could be that rice protein has already a high P.E. ratio as compared with the other three cereals.

A fresh experiment was carried out to compare the P.E. ratio of skim milk protein with cereal-pulse-amaranth mixtures. Skim milk gave a P.E. ratio of 2.568 ± 0.095 , the P.E. ratios of vegetable protein mixtures made up as above varied between 2.327 ± 0.064 and 2.472 ± 0.095 . These differences were statistically not significant indicating that dietary protein of high biological value can be obtained through vegetable sources.

Hæmoglobin and plasma protein in rats on vegetable protein diets.—Hæmoglobin and plasma protein levels in rats used in the previous experiments were determined after 4 and 8 weeks of continuous feeding of experimental diets.

The results of this experiment indicate that cereal-pulse-leafy-vegetable mixture can maintain normal levels of blood proteins.

Blood volume, plasma volume, hæmoglobin and plasma protein changes in rats in protein depletion.—It was felt that the influence of vegetable protein diets on the regeneration of plasma protein and hæmoglobin could best be studied in rats depleted in protein by dietary means alone.

As a preliminary step, blood volume, plasma volume, hæmoglobin and total plasma proteins were determined in 3 to 4 month old albino rats maintained on the colony stock diet. Blood volume was estimated by the Evan's blue (T 1824) dye standardized according to the method of Wang and Hegsted (*Amer. Jour. Physiol.*, **156** p. 227, 1949) with the modification introduced by Clusen and Lifsen (*Proc. Soc. Exper. Biol. Med.*, **91**, p. 11, 1956). The blood volume and body-weight of the rats showed the same relationship as had been observed by Wang and

Hegsted (*loc. cit.*) The regression equation for this relationship observed in our series of 107 animals was $y = 0.9897x - 1.1457$, where x is logarithm of body-weight and y that of blood volume.

Healthy rats from stock colony of an average age of 120 days were used for the experiment to study the changes resulting from protein depletion. Blood volume, p.c.v., plasma volume, hæmoglobin and plasma protein determinations were made on all animals which were then kept on a protein-free diet adequate in salts and vitamins. At the end of 4 and 8 weeks on the diet, similar determinations were repeated on some rats belonging to this group. Thus, two sets of determinations were available for each individual rat.

Certain interesting features of the results deserve comment. The body-weights decreased progressively till the 8th week remaining stationary thereafter till the 10th week. The blood and plasma volumes, on the other hand, remained unaltered at the end of the first four weeks. Although there was no change in the absolute values, the change in plasma volume and blood volume relative to the body-weight suggested a significant increase in these volumes. As a result, little change occurred in the total circulating amounts of blood proteins at this stage in spite of the decreases registered in the concentration of hæmoglobin and total plasma proteins. The real depletion in the circulating proteins became manifest at 8 weeks at which period the blood and plasma volumes registered a decrease in absolute values.

The effect of protein depletion and calorie restriction in albino rats.—Certain experiments dealing with this aspect have been described in the last Annual Report (1954-55, p. 8). The object was to determine (i) the effect on certain organs of protein depletion at various stages of deficiency and (ii) the influence of added calorie restriction on the effects of protein deficiency. The results have been subjected to a careful analysis and the conclusions reached are briefly described below :—

Pancreas.—The dietary level of protein influences the lipolytic activity of pancreas, it being lower at lower levels of protein intake. Calorie restriction alone has a similar effect although to a lesser extent. With the progress of time the effect of protein deficiency was maintained, while that of calorie restriction gradually decreased.

Liver.—Absolute liver weights were significantly lowered both in protein deficiency and calorie restriction, a marked effect being observed in combined deficiency. Protein depletion reduced significantly the total nitrogen per 100 g. of liver, whereas calorie restriction caused an increase.

The activity of liver transaminase and succinic dehydrogenase in protein inadequacy was significantly lowered, while under calorie restriction it was significantly raised.

Small intestine.—Protein deficiency lowered considerably the succinic dehydrogenase activity of the small intestine. The beneficial effect of calorie restriction superimposed on protein deficiency was seen throughout but was significantly greater on 42nd day.

It can be concluded from these experiments that calorie restriction, in general, affords some protection to the organs so far studied from the effects of protein depletion.

Protein deficiency and enzymes in rat.—A large number of rats of both sexes, 44 to 48 days old, and with the average weight of 82.5 g., were divided into six groups. Rats in the first five groups were fed diets containing protein at 15 (I), 10 (II), 7.5 (III), 5 (IV) and 2.5 (V), per cent levels, respectively. Rats in group VI received a 15-per cent protein diet, but the animals were paired with the group on 2.5 per cent protein in order to obviate the effects of reduced food intake which was observed only in this group. Observations on the activity of liver succinic dehydrogenase and xanthine oxidase were made by sacrificing rats at intervals up to a period of 60 days.

Rats in all the groups, except those receiving 2.5 per cent protein, gained in weight although to a different extent depending upon the level of protein intake. The liver nitrogen in rats fed 10 per cent protein and below decreased at 16 days; the decrease was greater at lower protein levels. After this period there was an increase in liver N with the result that in later stages of the experiment, in rats receiving 5 to 15 per cent protein liver N was not significantly different, whereas the liver N in rats on 2.5 per cent protein was significantly lower although it had increased from the value found in the same group at 16 days.

Succinic dehydrogenase.—The activity of this enzyme on 15 per cent protein diets showed a tendency to increase continuously till the termination of the experiment. In rats on 10 per cent protein the initial rise was followed by a fall at 16 and 24 days. As the experiment progressed, however, this fall was not only checked but a continuous increase till sixty days was observed. On the other hand, rats on 7.5, 5 and 2.5 per cent protein responded with a fall in activity below the initial level in the first two weeks at which period the fall was checked to be followed by a slow rise. There was a second fall at 48 days discernible in these three groups.

Xanthine oxidase.—Xanthine oxidase activity in rats on 15 per cent protein showed an increase throughout the course of the experiment. At 10 per cent protein level, the activity decreased for the first two weeks followed by a sustained increase although at a lower level than in group I.

At 7.5 per cent protein levels and below, xanthine oxidase activity was almost completely lost within the first eight days. The adaptation mechanism seen in the cases of liver N and succinic dehydrogenase was only partially evident in the case of xanthine oxidase. Among the rats on 7.5 per cent protein, livers of female rats only showed low activity at 48 and 60 days intervals, whereas no activity could be detected in livers of male rats in the same group. Livers of rats on 5 per cent and 2.5 per cent protein did not show any measurable activity up to 60 days for which period these experiments lasted. These results considered together with those on succinic dehydrogenase lead to the conclusion that the adequacy level of protein (in the form of casein) for rat would lie between 7.5 and 10 per cent. Levels below 7.5 per cent

protein the diet should, on account of the apparent failure of the adaptive mechanism, be considered clearly inadequate so far as the rat is concerned.

III. STUDIES ON FATS

(Under the auspices of the Council of Scientific and Industrial Research)

Identification and quantitative determination of iso-oleic acids in some natural fats.—The quantitative method of assay of mixtures of dibasic acids which was described in the previous report has further been extended to the investigation of the 'iso-oleic acid' fractions obtained from cow butter fat, beef body fat and hen egg yolk fat.

Butter fat and beef body fat.—The composition of the iso-oleic acid fraction arrived at by the application of this method established the presence in butter fat of Δ -7 Δ -8 Δ -9 Δ -10 Δ -11 and Δ -12 octadecenoic acids, and in beef body fat all of these except Δ -7.

Hen egg yolk fat.—It was not possible to obtain from hen egg yolk fat an 'iso-oleic acid' fraction which was solid at room temperature. From 200 g. of crude egg yolk fat, only 3 g. of 'iso-oleic' acids (I. V. 69.7) liquid at room temperature were obtained by mercuric acetate separation.

The results of chromatography of the dibasic acids obtained on the oxidation of this fraction (M.P. 103° to 106°) showed the presence only of azelaic acid indicating that the hen egg yolk fat was free from any other positional isomer of oleic acid.

IV. CHEMICAL COMPOSITION OF INDIAN FRUITS AND VEGETABLES

(Under the auspices of the Indian Council of Agricultural Research)

The chemical composition of a large number of fruits and vegetables used in India is given in Health Bulletin 23. There are however, several foodstuffs belonging to this category, about which no information was available. The purpose of the present investigation is to analyse the nutrient content of fruits and vegetables not listed in Health Bulletin 23. During the year under review, 40 fruits and 17 vegetables were investigated. The items analysed were: moisture, fat, protein, mineral matter which includes separate estimations of calcium, phosphorus and iron, and vitamins, such as carotene, thiamine, riboflavin, nicotinic and ascorbic acids.

The results of analyses have not been included in this report for lack of space. A few general comments are, however, given below:

Among the fruits thus far analysed Barhar (*Artocarpus lakoocha*) and giant capsicum are rich in vitamin C. All leafy vegetables and persimmon, and apricots among fruits are good sources of carotene. In general, leafy vegetables showed a good distribution of those components of vitamin B complex which were estimated in this investigation. Greens, dried fruits and some fruits of the hilly regions contained appreciable amounts of iron.

Special mention may be made of the leaves of several root vegetables, such as knol-kohl, beet, turnips, etc. which are usually thrown away. They are good sources of riboflavin, calcium, iron and carotene.

V. STUDIES ON ENERGY METABOLISM

We have little or no information in India on the subject of the energy cost of various physical activities in which Indian people engage in different walks of life. Such information is vital for arriving at the total calorie requirements of an individual or a group of people. With the intention of filling this gap in our knowledge, work was commenced during the last year on certain aspects of the problem. The portable Kofranyi-Michaelis calorimeter has been used in these investigations. Observations on the energy cost of a few activities were made under basal conditions, i.e. in fasting state 12 to 14 hours after a light meal the previous evening. The values obtained in cal./kg./hr. were as follows: Lying awake, 1.05; sitting on chair, 1.21; standing at ease, 1.30, and sitting typing 30 words per minute, 1.51.

Further work is in progress in which a variety of activities representative of various occupations will be investigated.

VI. RICE DIET AND FERTILITY

The results obtained in the short-term studies have already been described (*Ann. Rep.*, 1954-55, p. 17). The long term experiments are now almost completed. Neither in the prenatal period nor in litter size was there any significant difference between the animals maintained on 'wheat diet' and those on the 'rice diet' thus confirming the conclusion reached earlier that in rats there was no association between rice diets and fertility rate.

VII. NUTRITION CLINIC

During the period 1st April, 1955 to 31st July, 1956, 5,186 cases attended the clinic. Of these, 319 cases were found to be suffering from nutritional disorders.

VIII. CLINICAL INVESTIGATIONS

1. STUDIES ON PROTEIN MALNUTRITION

Hæmatological change in kwashiorkor.—A detailed hæmatological investigation of a series of 18 cases of kwashiorkor with ages ranging from 18 months to 5 years was undertaken. Owing to the absence of hookworm infestation and malaria in this region, it was expected that the hæmatological changes observed in cases of 'kwashiorkor' in Coonoor may be a more faithful reflection of the effect of uncomplicated malnutrition than in many other parts of the world.

On admission, all cases showed anæmia of moderate severity. M.c.h.c. ranged from 25 to 38 per cent with a mean of 31 per cent. The blood picture was macrocytic in 12 cases, normocytic normochromic in 4 cases.

and microcytic hypochromic in only two cases. There was no correlation between the severity of the anæmia and the extent of reduction in plasma albumin concentration.

Examination of the bone marrow showed three types of changes, e.g., normoblastic erythropoiesis with no abnormalities in the myeloid series in four cases; 'macronormoblasts' in addition to normoblasts in four cases; Ehrlich megaloblasts in addition to the normoblasts, macro-normoblasts and large metamyelocytes in nine cases.

Seven cases showed histamine fast achlorhydria, while eight others had no free acid in the resting juice or after alcohol meal. Cases with megaloblasts in the bone marrow were not necessarily those associated with histamine fast achlorhydria.

The response to treatment fell into two broad categories :

(a) Cases which showed appreciable improvement on treatment with high-protein diet, with or without iron; and (b) cases which did not improve with high-protein diet but which required folic acid or vitamin B₁₂, with or without iron, for their improvement. Cases showing Ehrlich megaloblasts in their bone marrow fell into the latter category.

The pace of improvement, particularly hæmoglobin regeneration, was slow in almost all cases including those with megaloblasts in the marrow and treated with folic acid or vitamin B₁₂. Appreciable improvement in gastric secretion was noticed in all cases after treatment with high-protein diet. Of seven cases which had histamine fast achlorhydria on admission, four responded to histamine after treatment.

Some aspects of hair changes in kwashiorkor.—Though there have been several clinical descriptions of the hair changes in kwashiorkor, attempts to correlate them with biochemical changes in the hair have been extremely few.

The subjects of this investigation included : (a) Six well nourished Indian children with black hair, (b) six African children, belonging to Uganda who were either suffering from, or had suffered from, kwashiorkor and who were in different stages of treatment. Two of them exhibited dyschromatrichia and the rest had apparently normal hair, (c) eleven Indian children belonging to Coonoor who were suffering from kwashiorkor. In cases in which segmental discolouration was observed, the brown and black segments of the hair were separately analysed, and (d) four Indian children belonging to Coonoor suffering from mild undernutrition but not kwashiorkor. All these children exhibited dyschromatrichia.

The amino acid composition of normal hair from well nourished Indian children was generally in close agreement with the values for normal hair reported in literature.

With regard to the six African children, there was a close association between the cystine content of the hair and duration of high-protein treatment, longer the period of such treatment the higher was the cystine content. The latter, however, bore no relation to the colour of the hair ;

the hair with the lowest and the one with the highest cystine content were both discoloured, while those with the intermediate values were of normal colour.

The Indian cases of kwashiorkor also revealed appreciable lowering of the cystine content before treatment and restoration after treatment. In cases with segmental discolouration, it was observed that the black segments had higher cystine content than the brown segments but the values were considerably lower than those obtained for the hair after treatment. The difference between the cystine content of the black portion of the hair obtained before treatment and after treatment was of a much higher order than the difference between the brown and black portions of the hair before treatment. Evidently, therefore, absence of dyschromatrichia did not necessarily signify normal cystine content. As regards amino acids other than cystine, there was no significant difference between the hair from normal children and those with kwashiorkor.

It can be concluded from those studies that the cystine content of the hair may be a better indication of the severity of protein malnutrition than the hair discolouration.

Further observations on the treatment of kwashiorkor.—In the present investigation which concerned a fresh series of 29 cases, observations on the effects of treatment on some biochemical changes have been presented. Cases in Coonoor had been treated successfully with 60 g. of protein intake daily. Dean (*Protein Malnutrition Conf., Proc. Jamaica, 1953, p. 212*), has, however, suggested a higher level of protein, i.e., up to 100 g. day for treatment. The question was whether such high levels of protein had any added beneficial effect. The results of the investigation may be briefly stated thus :—

(1) Comparison of the response to treatment with 60 g. and 100 g. of protein daily in two groups of cases of kwashiorkor revealed no significant difference with regard to serum albumin or clinical improvement. (2) Cases of kwashiorkor showed subnormal levels of serum sodium and calcium on admission. After high-protein diet treatment, the values rose to normal levels. (3) Serum potassium and magnesium concentrations in cases of kwashiorkor were within normal limits. (4) Prothrombin time was slightly elevated in cases of kwashiorkor on admission. The values returned to normal levels after treatment. (5) A number of cases of kwashiorkor which failed to show a satisfactory eosinopenic response to adrenalin on admission showed it after high-protein diet treatment.

2. STUDIES ON LACTATION

Fourteen mothers and their infants belonging to the poorest socio-economic groups were taken up for investigation immediately after the infants were born. Diet surveys indicated that the total calorie intake in these mothers ranged from 1,500 to 1,850 and the protein intake from 27 g. to 50 g. daily. The birth weights of the infants were accurately recorded on a sensitive lever-type balance. The actual amount of breast milk consumed by the infant in 24 hours was determined by hospitalizing mothers with their infants for 24 hours at a time at weekly intervals for

the first four weeks and at fortnightly intervals for the next 20 weeks. The infants were solely breast-fed for this period. The average body-weight was nearly double between the fourth and fifth month. The average 24-hour milk output ranged from 16 oz. to 18.6 oz., throughout the period of investigation and the average protein intake (calculated on the basis of 1.2 g. protein per 100 c.c. breast milk) of the infants ranged from 2 g./kg. body-weight in the first fortnight to nearly 1 g./kg. body-weight in the sixth month.

A comparison of the growth curves of the infants in the present study with those of normal American infants showed that the actual body-weights of the Indian infants at different periods were lower than those reported at the fiftieth percentile of American infants of corresponding ages. The two growth curves were, however, nearly parallel, signifying that though the actual weights were different, the growth rates were not widely divergent.

The results of the investigation suggest that the generally accepted figure for protein requirements in infancy of 3.5 g./kg. body-weight is probably an over-estimate and that the actual requirement may not be more than 2 g./kg. in the first few weeks and probably even less in the later stage.

3. EFFECT OF DIFFERENT CEREALS ON BLOOD SUGAR LEVELS

Indian dietaries are predominantly based on cereals, such as rice (raw or parboiled), wheat, ragi, and millets. Since these cereals do not differ widely in their carbohydrate (starch) content, their effect on blood sugar levels would be expected not to be significantly different. For a number of years, there has, however, been a strong clinical impression that patients with diabetes mellitus tolerate ragi (*Eleusine coracana*) better than rice and that their blood sugar levels and urine sugar levels tend to be lower on a diet based on ragi than on one based on rice. This impression has not been subjected to careful investigation.

The subjects were six normal male adults and two patients suffering from diabetes mellitus. The response to raw rice, parboiled rice, wheat and ragi was investigated in each subject at weekly intervals. The carbohydrate intakes in all the diets were identical and equivalent to that obtaining in six ounces of rice. The cereal was cooked and allowed to be eaten with 200 c.c. of butter milk and 3 g. of common salt. The patients were instructed to complete the meal within 15 minutes. Sugar was estimated in specimens of capillary blood of 0.1 c.c. obtained before the feeding and at 30 minute intervals after the commencement of the feeding for a period of 3 hours.

The differences between blood sugar levels following the administration of raw rice on the one hand, and those following on the administration of parboiled rice and wheat, on the other, were not significant. On the other hand, in all the eight cases investigated, there was a significant difference between the blood sugar responses following the feeding of rice and ragi. The differences were maximal and

significant with regard to the peak values obtained half-an-hour after the commencement of the test. This was true not only of the six normal subjects but also of the two cases of glycosuria.

The differences in the blood sugar response following on the feeding of the two cereals could also be obtained by feeding the respective starches, but were abolished when enzymatic hydrolysates of rice and ragi starches were fed.

4. NUTRITION AND CARDIOVASCULAR SYSTEM

Electrocardiographic studies on normal and malnourished monkeys (Macacus radiata).—In this investigation, attempts have been made to study the electrocardiograms of normal monkeys belonging to the species *Macacus radiata* and also the effects of calorie and protein malnutrition.

The present report of the electrocardiographic features of normal monkeys is based on 28 observations on 22 monkeys weighing between 3 and 15 lb. and recorded under pentothal anaesthesia. The animals were then fed a low-protein (3 per cent protein) diet for a period of about 6 weeks after which the electrocardiograms were repeated. On the completion of the experiment, the animals were sacrificed and their hearts and blood vessels examined histologically.

The electrocardiograms of normal monkeys showed heart rates varying from 140 to 260 beats per minute, P-R intervals from 0.06 to 0.14 seconds, QRS intervals from 0.03 to 0.06 seconds, and Q-T intervals from 0.12 to 0.24 seconds. The QRS axis angle varied from $+60^\circ$ to $+100^\circ$ in 18 monkeys from -70° to 114° in 9 monkeys. Unipolar limb leads in the latter indicated horizontal position of the heart. T_1 was flat in a majority of cases, while T_2 and T_3 were generally upright. TV_4 was upright in 17 cases, biphasic in 4 cases and inverted only in one instance. A noteworthy feature was the considerable normal variability with regard to several electrocardiographic features.

The most consistent change observed in the undernourished monkeys was a drop in the heart rate, which was noticed in all but 3 monkeys investigated. Changes consequential to the drop in heart rate were frequently observed in the Q-T intervals. The prolongation in Q-T interval in some cases, however, was greater than could be expected from the drop in heart rate. In five animals TV_4 which was initially upright, appeared inverted.

Myocardial and aortic changes in induced malnutrition.—Twelve monkeys were used for this investigation. A group of 4 animals was maintained on unlimited amounts of the colony stock diet containing 15 per cent protein. Eight animals were fed at 2 per cent protein diet for 65 to 78 days. At the end of the experimental period, the animals were sacrificed and a detailed autopsy examination performed.

The myocardium was normal in all 4 animals on the stock diet. In 3 of the 8 animals on the low-protein diet, however, evidences of myocardial damage were present. In the mildest form of the lesion,

isolated muscle cells were observed to have lost their cross-striations. In more severe types, larger numbers of muscle fibres were involved and they also showed splitting, fraying and fragmentation. That these bands represented newly formed connective tissue was shown by suitable staining procedures. The picture was one of diffuse interstitial myocardial fibrosis.

In monkeys on the stock diet, the aortæ were normal. In the deficient monkeys, on the other hand, striking changes were observed in the medial coat of the aorta. Varying grades of vacuolation of the interstitial tissue were intimately associated with a profound disorganization of the elastic tissue which was fragmented and thinned out and not arranged as closely packed rows of concentric lamellæ as in the normal aorta. Further, acellular masses staining metachromatically with toluidine blue and indistinguishable from mucoid were also seen. Abnormal accumulation of calcium in the media was demonstrated by the micro-incineration technique.

The lesions observed were thus similar in many respects to arteriosclerosis. Atherosclerosis characterized by lipid accumulation in the intima was, however, not observed in any of the monkeys kept on low-protein diets.

IX. PATHOLOGY

Investigations on the nutritional disorders of the liver have been continued. A long term study on the effects of partial replacement of rice in poor rice diets with tapioca flour has been completed and is described below. Investigations were also undertaken to study the influence of protein malnutrition on the structure of endocrine organs and the cardiovascular system in monkeys (*Macacus radiata*). Some of the results obtained have been described earlier in this report.

The effect of partial replacement of rice with tapioca on rat livers.—Subrahmanyam *et al.* (*Jour. Sci. Indust. Res.*, **9B**, p. 173, 195 ; *Brit. Jour. Nutr.*, **8**, p. 1, 1954) demonstrated that partial replacement of rice (25 per cent.) in poor rice diets by tapioca flour improved the growth of albino rats. They further advocated such replacement in Indian dietaries based on rice, particularly during times of cereal shortage. In view of the fact that replacement, even partial, of rice with tapioca would lower its total protein content, it was considered necessary to investigate the possible ill-effects of such a step.

In all, 192 young growing rats were used in this investigation, spread over three experiments. The diets which were based on rice were similar to those employed by Subrahmanyam *et al.* (1950). Early in the course of the investigation, it was realized that the rice and tapioca-rice diets differed in the calcium content, which was higher in the latter by about 10 mg. per 100 g. Hence, in the second and third experiments, additional groups of animals were maintained on the rice diet to which an equivalent amount of calcium phosphate was added. Growth rates of rats were studied during the first two months on the respective diets and systematic histological examinations of the livers carried out on batches of animals from each group that were sacrificed at interval extending up to 24 months,

In all the three experiments the growth of rats was found to be improved by partial replacement of rice with tapioca. Of much more significance was the remarkable increase in the weight of animals receiving calcium supplemented rice diet.

The pattern of histological changes in the livers of different groups of animals was comparable at any particular period of study. Histologically, the livers in all the groups showed mild to moderate degree of parenchymal damage of the protein deficiency type. At the earlier periods of observations the livers of the animals on the tapioca-rice diet showed these changes more often and somewhat more extensively. With progress of time (i.e. beyond one year), there was a partial regression of the lesions in all the groups of animals. Even at later intervals the animals on the tapioca-rice diet continued to show evidence of somewhat greater damage as compared with the other two groups. The calcium supplemented rice group, however, tended to return to normal one year onwards.

Thus, the results of this investigation show that partial (25 per cent) replacement of rice with tapioca does promote a better growth rate in young rats, by virtue of the higher calcium content. Supplementing the rice diet with an equivalent amount of calcium, in fact, caused a much more satisfactory growth rate. Histologically, however, the livers of animals receiving the tapioca-rice diet showed an accentuation of the protein deficiency types of liver damage.

X. FIELD INVESTIGATION

Protein malnutrition survey in South India.

An impressionistic survey of protein malnutrition in South India has been undertaken to ascertain the occurrence of this disorder and of the dietary and other contributory factors predisposing or leading to protein malnutrition. The results of surveys in certain areas in Madras, Mysore and Hyderabad State selected at random and completed up to the end of June 1956 are described below.

The sample and the scope.—The surveys were confined to a total of 2,657 children under 5 years of age, belonging to the poor sections of the community in rural and urban areas in five different areas in South India. The surveys consisted of :

(a) Recording of height and weight followed by clinical examination, (b) determination of blood hæmoglobin and plasma proteins in a certain proportion of children in each area, and (c) obtaining information on (i) occupation, income, living conditions, (ii) food habits, infant feeding and weaning, actual food intake by children, (iii) social customs and practices with regard to infant care and upbringing of children, and (iv) incidence of kwashiorkor in pediatric wards of the hospitals.

RESULTS

General aspects.—The occupations of the people in different areas were slightly different. In Hyderabad more than 80 per cent were

agricultural labourers with or without small land holdings, while in Mysore a good proportion were agriculturists. In Poonamalle and Madurai, the main occupation of the people was manual labour either in the fields or in the nearby textile mills. In Malabar, the group was almost similar, although it included nearly 20 per cent of fishermen. In spite of their slightly divergent occupations, their average income was not significantly different and was uniformly low. The environmental conditions and the personal cleanliness of the people examined were such that they would easily initiate and favour the spread of gastro-intestinal or other infections.

Care of infants and children.—Although the communities studied belonged to four different linguistic groups of Southern India, there were few differences in their practices relating to infant care and upbringing of children. The mothers from the poorer communities paid scant attention to feeding and care of their babies.

The commonest ailments of infancy and childhood were observed to be digestive disturbances and diarrhœa. This fact was recognized by the parents themselves, who, with a view to combating them had adopted certain practices which were more injurious to health than helpful.

General food habits.—The food habits of the people surveyed were simple and conformed to a uniform pattern in all areas except Malabar. Rice was the staple item of food and was occasionally replaced partially or completely by millets depending on the local availability. Vegetables, fruits, flesh foods, milk and milk products entered into the diets only occasionally. In Malabar, tapioca partly replaced rice. Instead of pulse-water, a preparation made from fish, coconut, spices and water was regularly taken along with the main meals.

Diets of pregnant and lactating women.—No special attention seems to be paid by the people to the diets of pregnant and lactating women. In fact, the wage earner is given preference over the pregnant women or children.

Infant feeding and weaning habits.—There was some uniformity in the infant feeding and weaning habits. Breast feeding was started invariably on the third day after birth because of the common belief that milk flow would start only three days after delivery. During this period, it was customary to get the baby fed by a wet nurse.

Breast feeding was always done on demand. In all the areas prolonged breast feeding was the rule although there were variations according to locality. In general it can be said that about 25 per cent of children were fully weaned at 12 months and about 25 per cent of the children still continued at breast even after 24 months from birth.

More than 75 per cent of the babies in Mysore and Malabar received supplementary feeds during the first year, while in Hyderabad, only less than 5 per cent got any supplements in the corresponding age group. In urban areas, there was a tendency to start supplements earlier.

Although the supplements were started at different times, in all the areas surveyed nearly 75 per cent children were completely weaned by the end of two years.

In Poonamalle, Madurai and Malabar animal milk of some kind was used in more than 50 per cent infants, whereas in Hyderabad and Mysore, cereals were given first to a majority of the babies. The quantities of milk wherever used, rarely exceeded 200 c.c. (7 oz.) a day, given mostly diluted with an equal volume of water. Milk feeds were gradually discontinued in favour of cereal foods.

In some of the children, who were still suckling, the quantitative intake of breast milk was assessed by the test-feeding technique.

The average output up to the end of 6 months was reasonably high (27 oz.) after which there was a gradual fall in the quantity which reached an average of 12 oz. day at the end of one-and-half years and continued at that level into the later periods of lactation. In some of the areas investigated, quite a number of children were solely on breast milk till one-and-half to two years. Obviously, this group was inadvertently exposed to severe restriction in dietary intake.

In all the areas, the consumption of both calories and proteins derived from breast milk as well as supplementary foods showed a striking reduction especially as the babies advanced in age. However, in Hyderabad, where supplementary feeding was delayed till the second year of life, the consumption of calories and proteins was relatively poor even in the first year.

A careful inquiry was made into the health conditions of the child and its siblings with particular reference to the occurrence of diarrhœa and œdema. A positive history of œdema associated with or preceded by attacks of diarrhœa at some stage of the childhood was obtained in more than 10 per cent of the siblings of the children examined. History of diarrhœa was elicited more frequently and several deaths from diarrhœa alone were also recorded in these areas.

Clinical examination.—Clinical examination with special reference to the various signs and symptoms of kwashiorkor was carried out on a total of 2,657 children.

No consistent differences could be observed in the incidence of various signs in the areas surveyed. Children, in general, looked undersized and unclean. Discolouration of hair, particularly at the tips and dry skin were observed more commonly in Pattancheru area, not only in children but also in some of the accompanying mothers. Enlargement of liver (more than two fingers below the costal margin) was observed more frequently in Madurai. Its incidence was rather low in Ramanagaram where relatively more cases of kwashiorkor were encountered. It would be interesting to establish the nature of the liver enlargement by biopsy or functional tests. Incidence of 'moon face' was relatively high in three of the areas. The significance of the above sign in the interpretation of protein nutritional status, is

not quite clear. Nevertheless, in spite of the fact that florid cases of kwashiorkor were generally removed to hospitals, a varying number of them was still encountered in all the areas visited.

Along with other deficiency signs, those of avitaminosis A predominated. Angular stomatitis was found next in order, while scurvy and active rickets were extremely rare.

Scabies in Malabar, coccal dermatitis in Pattancheru and acute or subacute conjunctivitis in Ramanagaram, were the special features frequently observed in the respective areas.

Somatometric data.—No significant differences were seen in the height and weight averages of the children from the different areas. Gross retardation in the growth of children in the communities studies was only too obvious.

Biochemical investigations.—Hæmoglobin, total serum proteins, albumin and globulin were estimated on a sub-sample of over a 100 children each from three of the five areas. Hæmoglobin levels were uniformly low in all areas and relatively more so in Pattancheru area. Total protein values seemed to fall within the normal range. However, on fractionation, albumin was found to be low, while globulins were high. The consistently low albumin values indicate an inadequate protein intake of prolonged duration. However, the explanation for an increase in the globulins is not quite clear.

Hospital statistics.—Personal communications from the pediatricians-in-charge of children's wards of hospital in Madras, Bangalore and Hyderabad, revealed that 16 to 10 per cent. of their in-patient admissions were cases of kwashiorkor.

PREVENTION OF PROTEIN MALNUTRITION

Test trials with protein-rich foods.—It is now generally accepted that the problem of protein malnutrition in children must be tackled on the preventive side. Controlled observations in the clinic had shown that Bengal gram, suitably processed, could be used to treat kwashiorkor (*Ann. Rep.*, 1953-54, p. 14). It was felt that a combination of four parts of roasted Bengal gram flour, 1 part of skim milk powder and 0.5 part of jaggery would constitute an economic palatable and effective preparation as a supplement for the preventive approach. About 15 g. of protein could be given at one sitting to the children through this preparation. A pilot study was, therefore, undertaken to determine its acceptability. The mixture of roasted Bengal flour, skim milk and jaggery in proportions mentioned above was mixed with water just enough to enable it to be made into soft balls. The average weight of the preparation (one ball) was 33 g.; it provided 7.5 g. protein and 120 calories. About 30 children under 5 years of age from a village in the vicinity of Coonoor, were given 1 to 2 balls daily according to age, regularly for four months. In a few cases, transient diarrhoea was complained of but it disappeared soon without any treatment. No child discontinued taking the preparation because of this minor episode. Subsequent consumption of the same for a few months did not produce any unusual symptoms. Field trials on a larger number of children in a controlled study are now contemplated.

2. Nutrition Research Unit under Dr. B.C. Guha, at the University College of Science & Technology, Calcutta.

I. STUDIES ON THE BIOSYNTHESIS OF ASCORBIC ACID.

A. *In vitro* experiments with pyruvate on the biosynthesis of ascorbic acid.

In vitro experiments with homogenized rat-liver tissues and with tracer technique further confirm that pyruvic acid is a precursor for the synthesis of L-ascorbic acid by the rat.

The homogenized tissue was incubated at $37.5 \pm 0.5^\circ$ for 3 hours under various experimental conditions, using sodium pyruvate as the substrate. It was found that as that tissue concentration increased the amount of ascorbic acid synthesized decreased and vice versa. Conditions have been determined under which 70 to 100 per cent increase in ascorbic acid can be brought about and these are summarized below :

Tissue : medium (during homogenization)	1 : 4
Tissue concentration (during incubation)	50 mg/c.c.
Substrate concentration	 0.05 M pyruvate
Buffer concentration	 $\frac{M}{15}$ = Sorensens phosphate buffer
pH	 7.2
Total volume of the mixture	... 10 c.c.

Chlorotonized and starved rat-liver tissues always gave better conversion of pyruvic acid than those from non-chloretonized and unstarved rats. Differences in sex, age and weight did not affect the biosynthesis *in vitro* materially. *In vitro*, chloretonized male rats however, excreted considerably more ascorbic acid than females.

Pyruvic acid had no protective action on L-ascorbic acid in presence or absence of rat-liver tissues under the experimental conditions studied. The observed increase in ascorbic acid could not, therefore, be due merely to its protection from oxidation. That the product of incubation of pyruvate was L-ascorbic acid only and no other indophenol reducing substance was proved by paper chromatographic analysis of the incubated extract, where only one spot having the R_1 of pure L-ascorbic acid was detected. This was further confirmed by incubation of 2- C^{14} pyruvate, and identification of C^{14} -L-ascorbic acid on a paper chromatogram. The quantity of C^{14} -L-ascorbic acid formed from 2- C^{14} -pyruvate was estimated by scanning the chromatogram. It had been found that about 2 per cent of the C^{14} pyruvate had been converted into C^{14} -L-ascorbic acid.

B. FACTORS INFLUENCING THE BIOSYNTHESIS OF ASCORBIC ACID.

In previous reports it has been shown that the biosynthesis of ascorbic acid takes place when the liver of chloretonized rats is incubated

with pyruvate. It has also been shown that the injection of ATP and malic acid abolishes this increased synthesis of ascorbic acid. In order to know which cell components are responsible for the synthesis of ascorbic acid, experiments were carried out to study the effect of the injection of carbon tetrachloride on ascorbic-acid synthesis, which specifically attacks the mitochondrial fraction of the cell components, and also disorganizes the tricarboxylic acid cycle as observed by Judah and Christie (*Proc. Roy. Soc.*, **B**, **142**, 241, 1954). The results of the experiments show that carbon tetrachloride has got some definite depressing effect on the urinary excretion and the ascorbic-acid content of the liver of the chloretonized rat. It appears, therefore, that the mitochondria are involved in the synthesis of ascorbic acid.

Further observations have been made to throw some light on the probable pathways of the biosynthesis of ascorbic acid. Experiments have been performed to see whether the enhanced synthesis of ascorbic acid under chloretone is interfered with in alloxan diabetic rats. The results show that the diabetic rats maintain an enhanced synthesis of ascorbic acid under chloretone. This seems to indicate that hexokinase of the normal glycolytic system which is impaired by alloxan diabetes does not play an important role in the biosynthesis of ascorbic acid.

II. BIOCHEMICAL STUDIES ON NICOTINIC ACID.

This year the work has been directed to isolate the bound nicotinic acid (BNA) from the rice bran extract with the help of ion-exchange resins, and cellulose powder column chromatography. In course of this investigation, a suitable chemical method has been developed for the estimation of bound nicotinic acid.

It is known that both free and bound nicotinic acid (BNA) produce coloured compound in presence of CNBr and *p*-amino benzoic acid (PABA). It has been observed that the coloured compound produced by BNA could not be extracted with any of the organic solvents tried, such as amyl alcohol, butanol, ether and ethyl acetate, although the coloured compound formed with free nicotinic acid is extractable with amyl alcohol as observed by Swaminathan. This observation has been used to estimate BNA in presence of free nicotinic acid in a given sample of rice bran.

In order to isolate the BNA, different ion-exchange resins, such as IR-100, IRA-410, decalso, etc. were tried but the results of the experiments so far obtained are not very encouraging. Recently, attempts have been made to separate BNA on cellulose column. Preliminary observations on cellulose column appear to give better results.

III. STUDIES ON IRON METABOLISM.

Studies by means of paper electrophoresis revealed that there are two haemoglobin components, I and II, present in the red-cell hæmolysates as obtained from the chick. Studies of the effect of age and diet

showed that, with the increase of age, the component I increases while component II decreases. Paper electrophoresis of hæmoglobins obtained from other avian species such as pigeon, duck, guinea-fowl also revealed the two hæmoglobin components as in the case of chick. These hæmoglobins were not identical with the hæmoglobins of mammalian species, viz. the guinea-pig, pig, cow, goat and rabbit. Component II hæmoglobin of avian blood has been found to be identical with Hb.E. of the blood of thalassemic patients.

IV. STUDIES ON THE INCREASE OF ASCORBIC ACID OF SOME VEGETABLES DURING BLANCHING AND OF RIBOFLAVIN DURING STORAGE.

(1) It was reported last year that there was an increase of ascorbic acid in cauliflower during blanching. The result was confirmed this year too. Slight increase of ascorbic acid was also observed in spinach and turnip but not in radish and potato. Since interest in the bound form of ascorbic acid (ascorbigen) from which ascorbic acid is released by heat in an inert atmosphere (Guha and Sengupta, *Nature* **141**, 974, 1938) has recently been revived by other workers and Prochazka has claimed its isolation. Attempts have been made to separate this substance from dried amla powder and from cabbage juice.

(i) Evidence for a bound form in amla was obtained and it was also possible to adsorb it on activated carbon (E. Merck) but it was not possible to elute it with chloroform-alcohol mixture or with ethyl acetate.

(ii) Work on cabbage has proved more promising. A concentrate from cabbage extract has been obtained which when heated with acid gives an increase in ascorbic acid values from 2.4 to 48.4 mg. The bulk of it could be adsorbed on activated carbon. Of this a little less than half has been eluted with chloroform-alcohol mixture. Preliminary chromatographic experiments indicate the identity of the indophenol reducing substance released from this partially purified bound form to be ascorbic acid.

(b) The use of trypsin at pH 7.8 instead of papa in-take diastase at pH 4.5 for the liberation of riboflavin in samples gave definite increase in the riboflavin content of skim-milk-powder and Bengal gram. The increase in the riboflavin content of canned parwar (*Trichosanthes dioica* Roxb) and peas (*Pisum sativum*) to an extent of 80 per cent and 90 per cent, respectively, during storage for 6 months was reported earlier. It appears that in estimation by standard methods, riboflavin is not wholly liberated from the sample. Its enzymatic liberation deserves further study.

V. STUDIES ON THE RETENTION OF ASCORBIC ACID DURING BLANCHING OF FOODSTUFFS.

Attempts were made to prevent the loss of ascorbic acid during blanching of some vegetables prior to canning. Methods tried were (1) blanching in water, (2) blanching in 0.11 per cent Na_2SO_3 (anhydrous), (3) dipping 0.5 per cent Na_2SO_3 for 30 seconds and then blanching in water, (4) dipping in 0.075 per cent Na_2SO_3 + 5 per cent NaCl for

1.5 min. and then blanching in water, and (5) blanching in acid medium (lactic acid) pH 3.5. All the samples were balanced in 3 volumes of water or any other appropriate solution for 4 minutes at 82° C.

The vegetables studied were parwar (*Tricosanthes dioica* Roxb) cabbage (*Brassica oleraceacapitata*), spinach (*Spinacia oleracea*), (bitter-gourd (*Momordica charantia*) and kankrol (*Momordica cochinchinensis* spreng).

It was observed that all the methods (2-5) could retain more ascorbic acid than when the samples were blanched in water, the effect being the greatest with lactic acid (5) and sulphite blanching (2).

VI. THIAMIN VALUES OF PURE-BRED STRAINS OF RICE.

The thiamin values of pure-bred strains of rice obtained from 1952-53 and 1953-54 harvests were reported earlier. The same strains obtained this year from the respective agricultural research stations of the different State Governments and also from the Central Rice Research Institute and the Indian Agricultural Research Institute were analysed for their thiamin contents. It was noted that the thiamin values of the strains of rice obtained from this year's harvest did not differ markedly from the values obtained for those strains of previous harvests. It was also noted that thiamin contents of different strains from the same station differ widely, though they were grown under similar conditions. This seems to support the idea that the thiamin content of a particular variety of rice is a characteristic of that variety. It is suggested that those strains of rice whose thiamin contents are high should be cultivated at least in those areas where they normally grow provided they are up to the mark in other important qualities, e.g. yield, resistance to diseases, etc.

VII. RIBOFLAVIN AND NICOTINIC ACID CONTENT OF PURE-BRED STRAINS OF PULSES.

Pure-bred strains of pulses obtained from the agricultural departments of the different State Governments were analysed for their riboflavin and nicotinic-acid contents. Values differ widely amongst varieties of any particular pulse obtained from the same or different agricultural stations.

3. Nutrition research unit under Dr. S.K. Gokhale at the Seth G.S. Medical College, Bombay.

During the year under report work on the nutritive value of pure bred strains of cereals and pulses was continued. Estimation of riboflavin, nicotinic acid and folic acid have been carried out on certain pure bred strains of paddy, wheat, jowar and certain pulses. Samples of seeds from successive years' crops, and from different cultivation methods, like the Japanese, local and departmental, and samples from specific manurial and soil experiments and seed colour variations from certain Agricultural Research Stations have been obtained and are

being examined. Studies on the effect of dietary components on the maintenance of folic acid, citrovorum factor ratio and its correlation with the proximate principles of food in the rat liver have been undertaken. Results obtained so far showed that increase in the dietary protein caused in the liver an increase of folic acid, folinic acid and also ascorbic acid and also folic acid to folinic-acid conversion. In the case of high fat and high CHO diets no significant effect on the conversion of folic acid to folinic acid was observed. It was also seen that decrease in the liver protein is correlated to the reducing capacity of the liver to store vitamins. Studies regarding the liberation of amino acids from cereals and pulses is being continued. Estimations of total pyridine nucleotides in blood of normal subjects has been continued. An investigation has been also undertaken to estimate iron and copper in serum and its fractions in normal subjects.

4. Inquiry into metabolism of vitamin A at the All-India Institute of Hygiene & Public Health, Calcutta.

Isomerised linseed oil and isomerised linoleic acid were found to have some protective action on vitamin A-deficient rats. This problem was further investigated to elucidate the nature of this protective action. It was found that these substances could only prolong the life of the vitamin A-deficient rats by about 10 to 14 days, but they could not relieve the deficiency symptoms at all. It was also found that when these substances were added to vitamin A-free diet, the depletion period for vitamin A was prolonged. In this respect they have got a similarity of action to α -tocopherol, which was simultaneously given to another group of rats. Most probably they act as a protective agent for vitamin A.

The water soluble complex of albumin and retinene was studied by paper electrophoretic method. Two more parallel investigations were done—one with plasma albumin, in which vitamin A alcohol was dispersed and the other with plasma from rats, given excessive amount of vitamin A by mouth. In the first case, it was found that the albumin fraction moved forward, leaving the vitamin A stationary, but in the second case vitamin A moved with protein fractions.

Tissue respiration studies with organs of vitamin A-deficient rats and hypervitaminotic A rats are in progress.

5. Inquiry on metabolism of nicotinic acid under Dr. S. Banerjee at the Presidency College, Calcutta.

The dietary tryptophan is converted into nicotinic acid in the body of the rat. While some workers claim that this transformation occurs in the intestine by the activity of intestinal micro-organisms, others have shown that it takes place in the tissues of animal. It was shown in previous reports of this inquiry that liver of rats was particularly concerned in this transformation. Later, it was observed that monkeys also synthesize nicotinic acid from tryptophan. The site of synthesis of nicotinic acid from tryptophan had so far not been studied in *rhesus*

monkeys. Metabolites of nicotinic acid, such as nicotinuric acid, quinolinic acid and N'-methyl nicotinamide, were estimated in the urine of *rhesus* monkeys before and after they were fed dl-tryptophan. The experiments were repeated after the animals were fed sulfaguanidine or chloromycetin. These metabolites were also estimated in urinary excretions of monkeys whose liver had been damaged by injections of carbon tetrachloride both before and after they were fed dl-tryptophan.

These excretions were diminished when the monkeys received sulfaguanidine or chloromycetin. Carbon tetrachloride injections did not affect the urinary excretions of these metabolites. This indicated that intestinal flora is possibly concerned in the synthesis of nicotinic acid from tryptophan.

It has been found that deficiency of pyridoxine in dogs and mice gives rise to an increase in the urinary excretion of xanthurenic acid and there is a consequent decrease in the conversion of tryptophan to nicotinic acid. It has further been reported that rats when fed nicotinic acid excrete increased amounts of ascorbic acid in the urine and it has been claimed that symptoms of B vitamin deficiency in rats could be prevented by feeding them with ascorbic-acid. The effect of feeding pyridoxine and of withdrawal of ascorbic acid supplements on the urinary excretions of nicotinic-acid metabolites before and after dietary supplementation with tryptophan was also studied in *rhesus* monkeys.

Nicotinic-acid metabolites were excreted in larger amounts when the animals were fed pyridoxine. Vitamin C deficiency did not affect the excretions of these metabolites. This showed that pyridoxine helps in the transformation of tryptophan to nicotinic acid and that vitamin C is not concerned in the synthesis of nicotinic acid from tryptophan.

Biosynthesis and metabolism of nicotinic acid in rats.—It was previously reported by us that when liver of rat was poisoned by carbon tetrachloride, the animal could not synthesize nicotinic acid from tryptophan. It was suggested that liver was the site of nicotinic-acid synthesis in rats. The effect of feeding methionine to rats whose liver had been damaged by carbon tetrachloride on the urinary excretion of nicotinic-acid metabolites was studied. It was observed that poisonous effect of carbon tetrachloride on liver could be counteracted by methionine and the capacity of liver to synthesize nicotinic acid from tryptophan could be restored to normal.

6. Inquiry on studies on energy metabolism under Dr. S. Banerjee at the Presidency College, Calcutta.

Preparation of a nomogram for the calculation of the surface area of Indian male subjects.—From the previous work it was found that the universally accepted formula of Du Bois and Du Bois for the calculation of the surface area of the body is not applicable in the case of Indian males and the new formula

$$\left\{ \begin{array}{l} \text{SA} \\ (\text{cm.})^2 \end{array} = \frac{\text{H}^{0.725}}{(\text{cm.})} \times \frac{\text{W}^{0.425}}{(\text{kg.})} \times 74.66 \right\}$$

was suggested. In order to simplify the tedious calculations for the

above formula a nomogram was prepared this year. If the height and weight of the person in metric or British units are known, the surface area in square meters of the person can easily be found out from the nomogram by joining the points representing the height and weight by a line which crosses the scale of surface area at the required value.

Determination of the respiratory quotient of Indian females under basal condition.—To find out the normal BMR level of the Indians under different environmental conditions, 50 male subjects were studied last year. This year 75 female subjects, mostly college girls, were studied. With the help of Douglas bag, Haldane-Henderson-Bailey gas analysis apparatus and a gasometer, the volume of oxygen consumed and carbondioxide produced were determined and respiratory quotient (R.Q.) was calculated. In Indian females the R.Q. varied from 0.74 to 0.98. The average R.Q. was 0.83.

Determination of the respiratory quotient of Indian males under basal conditions.—The respiratory quotient of another 30 normal Indian male subjects under basal condition was determined.

Their R.Q. in basal condition varied from 0.72 to 0.98 and the average R.Q. was 0.84.

Determination of the basal metabolic rate in 75 normal Indian females during the winter, summer and rainy seasons by the Collins-metabolex apparatus and also by the Douglas-bag method.—The basal metabolic rates of 75 normal Indian females were determined in the winter, summer and rainy months both with the Collins-metabolex apparatus and by the Douglas-bag method. The room temperature varied between 81°F. and 95°F. The humidity varied from 31 per cent to 84 per cent. The B.M.R. was taken on the 10th to 20th day after the last menstruation. The age of the subjects varied from 9 years 3 months to 46 years, the height varied from 119.2 cm. to 162.2 cm. and the weight varied from 18.03 kg. to 62.60 kg. The average basal mouth temperature was 98.6°F. and the average basal pulse and respiration rate per minute were 72 and 17, respectively.

The oxygen consumption was measured with the metabolex apparatus in the usual manner. From the oxygen consumption the total heat production in 24 hours was calculated. The surface area of the body of the subjects was calculated by the use of our new height and weight formula and the B.M.R. in calories per square meter of the body surface per hour was calculated.

The total heat production in 24 hours under basal condition varied between 751 and 1,279 calories. The average is 1,031 calories. The B.M.R. varied from 25.80 to 35.00 calories per square meter of the body surface per hour. All these values were low in comparison with the Western standards. In comparison with the Mayo Foundation Standards the B.M.R. varied from 1 to 25.6 per cent.

With the Douglas-bag method, it was found that the total heat production in 24 hours varied from 699 to 1,353 calories, the average being 1,093 calories. The B.M.R. varied from 25.50 to 38.43 calories per hour per square meter of body surface, the average being 31.56 calories/hr./sq. m. which was 14 per cent too low in comparison with

the Mayo Foundation Standard. In most of the cases the B.M.R. values with the Douglas bag method were slightly higher than those obtained with the metabolex apparatus.

7. Inquiry on vitamin amino-acid interrelationship with special reference to folic acid and vitamin B₁₂ under Dr. A. Sreenivasan at the Department of Chemical Technology, University of Bombay, Bombay.

Biosynthesis of choline.—Work was continued on the relationship of folic acid to choline metabolism along three lines : (a) serine metabolism, (b) metabolic changes on a low-choline low-methionine diet, and (c) phospholipid synthesis in *Neurospora*.

(a) *Serine metabolism.*—A study of the intracellular distribution of serine decarboxylose, serine dehydrase and glycine serine-systems was completed. The general effects of dietary deficiencies of vitamin B₆, biotin and folic acid on liver nitrogen and total amino acids were also ascertained.

The transformation of glycine to serine and of folic acid to citrovorum factor by rat liver enzymes was inhibited by 40 and 50 per cent respectively, in niacin deficiency, supporting the postulated involvement of DPN in transformylations, and more specifically in folic acid to citrovorum factor conversion.

(b) *Metabolic changes on a low-choline low-methionine diet.*—In rats, reared on a five per cent casein diet deficient in choline, methionine and folic acid, negligible stores of liver phosphorylcholine were observable except with supplementation of both folic acid and choline. This would suggest that, for folic acid to function in phosphorylcholine biosynthesis, adequate choline supply is necessary.

The conversion *in vivo* of p-aminobenzoic acid to p-aminobenzoyleglycine and the *in vitro* esterification of inorganic phosphate were both affected in folic-acid deficiency. Contrary to expectation, however, methionine oxidation was less in folic-acid deficiency. It is possible that any increase in methionine to formate conversion in folic-acid deficiency is marked by decreased formate oxidation. It is necessary to study methionine to formate conversion in relation to dietary folic acid.

A study of fat absorption in relation to dietary choline and folic acid showed an expected increase on choline supplementation. This was, however, so even in folic acid supplemented groups, the effect being more in presence of choline. Work is in progress to ascertain whether the influence of folic acid is explicable on the basis that fat transport after absorption is interfered with in folic-acid deficiency causing an unfavourable gradient.

(c) *Choline synthesis in Neurospora crassa.*—Synthesis of choline from ethanolamine and methionine was shown in homogenates from washed mycelia of the wild strain of *N. crassa*. Both the particulate and soluble supernatant fractions of the homogenate were necessary for the

conversion, the factor in the supernatant being heat-stable. In preliminary work, this organism has also been observed to synthesize methionine from homocysteine and serine.

(2) *Vitamin B₁₂ and oxidative metabolism :*

(a) *Thyrotoxicosis*.—In hyperthyroidism there was an altered distribution of vitamin B₁₂ in liver cell fractions attributable to osmotic damage in mitochondria.

(b) *Experimental liver damage*.—The observations on vitamin B₁₂ protection in CCl₄ toxicity were extended to a study of centrifugally separated cell fractions. Changes in total and phospho-lipids in steatotic livers were confined to the supernatant fraction, the nuclei and mitochondria being unaffected.

Uncoupling of oxidative phosphorylation demonstrated in experimental hepatic injury by CCl₄ administration was correlated with the lability of intramitochondrial E₂₆₀ (nucleotide) components. In mitochondria from steatotic livers, the initial release of E₂₆₀ material was considerably low and pointed to a damage to mitochondrial morphology *in vivo*. Protection by vitamin B₁₂ against CCl₄ poisoning was reflected in the initially high release of E₂₆₀ material as well as its more normal increased release with time into the surrounding medium. This protection might arise from the known influence of the vitamin on sulphydryl conservation and nucleotide metabolism.

(c) *Sulphydryl metabolism*.—Reference was made in the last report to a study of the rate of incorporation of administered cysteine into liver sulphydryl compounds in vitamin B₁₂ deficient and in hyperthyroid rats in comparison with vitamin B₁₂ fed groups. Further studies with normal rats showed that cysteine administration resulted in maximal values for total sulphydryl compounds in liver after 3 hours when only 50 per cent of this was present as glutathione. Normal total sulphydryl levels were reached after about 24 hours. The glutathione concentration steadily increased during 12 to 14 hours reaching normal levels after 24 hours. Prior administration of vitamin B₁₂ did not make any difference to this pattern.

The observation that liver glutathione is present only in nuclear and supernatant fractions, the former being relatively static in conditions of stress such as hyperthyroidism and steatogenic poisoning, was extended by attempts at *in vivo* synthesis of glutathione in the perfused liver and *in vitro*, with isolated nuclei.

8. Inquiry on the influence of cooking on the nutritive value of foods under Dr. M.L. Pai at the Medical College, Baroda.

Synopsis of the work and results achieved.—There is a wide variation in the nutritional content of foods before and after cooking. Many of the articles of food are subjected to the varying types of processes of cooking before they are eaten. The chemical analysis of the raw articles of food may sometimes be misleading as guide to its nutritional

value, because some of the material estimated in the raw article, may not be available for nutrition, after it has been subjected to the varied changes during the process of cooking.

Line of work.—The analysis with respect to the estimations of nicotinic acid, riboflavin, thiamine, ascorbic acid, vitamin A (when present) and those of calcium, phosphorus and iron are carried out in half the portion of the sample of the raw material and then in the remaining half of the portion which is cooked.

Work done.—Since the time of the report submitted for the last year, i.e. from October 1955 to September 1956, work on the above lines has been in progress with regard to the analysis of other items of food preparations as follows :—

1. These preparations included vegetables, e.g. radish, luffa, aegyptrea, vegetable marrow, etc., pulses, such as the dahl of field-bean, etc., legumes, such as dry peas, etc.

2. The preparation, such as the 'khaman' is commonly taken by the people here. This is prepared out of gram-dahl, allowed to ferment overnight.

3. Analysis of some of the items of the hospital diet served to the patients in the wards, e.g., preparation of rice and chapati, etc.

4. The above preparations, except the preparation such as khaman which was brought from the shop, were studied for their chemical analysis both in their pre-cooked and cooked stages, under the controlled conditions of the laboratory, where the preparations were actually cooked.

5. Articles, such as radish, dry peas, Bengal gram and lentil were analysed in various stages, namely (i) raw, as obtained in the market, and (ii) pre-cooked, i.e. raw article which was subjected to the treatment of washings, etc. Then the other half which was left after the article was taken for its analysis in the pre-cooked stage was divided in three portions to be cooked after three different types of cooking procedures which were as follows :—

1. Ordinary cooking by application of direct heat to the cooking utensils.

2. Steam-cooking, i.e. cooking in the ordinary cooker made of cooking vessels of the type of tiffin-carrier which are placed one above the other.

3. Steam cooking in pressure cooker.

The results.—The results obtained from the analysis of the preparations described above have confirmed the previous finding. Thus, the results have shown that the losses of the vitamins, such as vitamin C, thiamine, riboflavin and nicotinic acid in the preparation which have been subjected to the prolonged heating or boiling, at high temperature, are more than those in the cases of preparations where they have not been subjected to such prolonged heating, at high temperature but instead to heating for a short interval of time at a lower temperature.

Furthermore, some interesting results have been obtained when the articles were cooked by three different types of cooking procedures as outlined above. The losses of the vitamin studied were more in the first type, i.e. cooking by direct application of heat to the utensils than those in the case of the other two types of cooking procedures, i.e. cooking in ordinary cooker or pressure cooker.

9. Inquiry on studies on the intracellular distribution of vitamins under Dr. J. Ganguly at the Indian Institute of Science, Bangalore.

Vitamin A esterase.—The distribution of vitamin A-esterase activity in various tissues of the rat was studied with vitamin A acetate, stearate, palmitate and oleate. Liver was active against the acetate only, while pancreas, small intestine, spleen and kidneys were active against all the four esters and blood plasma showed slight activity against the acetate. Red blood cells were completely free of activity against all the four esters of vitamin A.

Rat liver vitamin A-esterase showed two pH optima at pH 6.6 and at pH 8.6, the maximum activity being obtained at pH 8.6. The substrate optimum of the liver enzyme was at a concentration of $6 \times 10^{-4}M$ above which concentration the activity decreased sharply with increasing concentration.

Various metallic ions, a few alkaloids and arsenicals were tested for their effect on the activity of the liver enzyme with respect to vitamin A acetate and ethyl butyrate as substrates. Many of these agents, preferentially inhibited vitamin A-esterase activity, while having no effect on the esterase activity. Acetone drying of the liver caused almost complete loss of vitamin A-esterase activity, while retaining most of the esterase activity. The vitamin A-esterase activity of rat liver homogenate on aging at $5^{\circ}C$ went down rapidly from the sixth day onwards, whereas the esterase activity was retained almost unchanged even up to the fifteenth day. Heat inactivation studies with liver homogenate showed that the vitamin A-esterase activity was rapidly lost at $50^{\circ}C$, while the esterase activity was retained up to $65^{\circ}C$. These studies indicate that the vitamin A-esterase activity was more labile than the esterase activity, and it is, therefore, possible that the two enzymes are not identical.

On wet-weight basis chicken liver was more active than rat liver. While in the rat liver the activity was clearly localized in the microsomal fraction (as obtained by differential centrifugation) in the chicken liver, using identical techniques as used for rat liver, the activity was widely distributed amongst the nuclear, mitochondrial, microsomal and supernatant fractions. This might be indicative of a species difference in the intracellular distribution of vitamin A-esterase activity.

Homogenates of livers containing large amounts of stored vitamin A (mostly in the esterified form), when incubated alone at pH 8.6 showed no hydrolysis of the vitamin A ester, but addition of pancreas brought about rapid hydrolysis. Small amounts of vitamin A ester could be detected in starving rat blood, and homogenates of pancreas, kidneys

spleen and small intestine were able to hydrolyse this vitamin A present in blood. It is possible that the small amount of ester present in the circulating blood is hydrolysed by the tissues, such as pancreas, kidneys, spleen, small intestine, etc., and thus the vitamin A-alcohol levels in both blood and liver are maintained.

10. Inquiry on studies on metabolism of starvation and malnutrition under Dr. M.L. Chakraborty at the Medical College, Calcutta.

Eighty cases of malnutrition (human subjects) and six cases of guineapigs in whom malnutrition had been produced experimentally were studied and a fall in the levels of sodium, calcium and magnesium was noted. The fall in the latter two items is, however, small and that also occurs much later than the fall in the level of sodium. The serum potassium attains a high normal value. The low normal value of serum, sodium and high normal value of serum potassium in conditions of malnutrition suggests hypo-function of supra-renal cortex. X-ray of femur showed osteoporosis. The urine examination revealed a greater excretion of calcium. These two observations suggest bony depletion of calcium in conditions of malnutrition. Simultaneously with the fall in the levels of sodium, calcium and magnesium there are also diminutions of blood sugar, cholesterol, urea N.P.N. A few healthy cases (30 in number) under similar conditions were investigated to blood picture, which will give an idea as to how the comparative deficiency of hæmatological and biochemical items are responsible for the production of altered clinical picture.

11. Studies on the growth and physical development of Indian infants and children under Professor D.N. Mazumdar at the Lucknow University, Lucknow.

To form norms of growth for Indian children between the ages 0 and 20 years a cross-sectional sample survey has been attempted. India for this purposes has been divided into five zones. We are covering the North Zone comprising of Uttar Pradesh, Delhi, Punjab, PEPSU, Himachal Pradesh, Bilaspur and Jammu and Kashmir. 0.1 per cent of the total population between the ages 0 and 20 years has to be covered. The effect of certain factors on growth is being studied. The factors and their classifications are given below :—

Geographical Division.—Same as 1951 census classification.

Rural and urban classification : Rural.—

(a) If an individual lives in a village.

(b) If he has been living in a village for more than 60 per cent of his age, otherwise Urban.

Religious Distribution.—The three main groups to be considered will be Hindu, Muslim and Christian,

Economic Distribution.—The classification will be with regards to income per month as :

1. Rs. up to	Rs. 50/-
2. Rs. 51/-	Rs. 100/-
3. Rs. 101/-	Rs. 200/-
4. Rs. 201/-	Rs. 400/-
5. above Rs 401/-	

Occupational Distribution.—The groupings are as follows :

1. Executive service (pay above Rs. 250/- p.m.)
2. Services other than 1.
3. Business.
4. Landlord and farmers.
5. Professions (doctors, lawyers, teachers, priests)
6. Retired.
7. Unemployed.
8. Cultivators of owned lands.
9. Labourers.

Dietary Distribution.—The classification will be :

1. Vegetarian.
2. Non-vegetarian.
3. Mixed.

Linguistic Distribution.—The classification will be made according to the mother tongue of the individual.

Environmental Distribution.—The groupings are as follows :

1. Pukka house with good sanitary facilities.
One room occupied by two or less adults.
2. Pukka house. One room occupied by three or more adults.
3. Kuccha house.

SAMPLING TECHNIQUE ADOPTED.

The total sampling units has been distributed among the seven component states of North Zone in the ratio of their population. Assuming that the district within a geographical division of the States (1951 census) are homogeneous 40 per cent of the districts within each of these geographical zones of the State have been selected at random with equal probability. The sampling units of the geographical division have been distributed among the selected districts in the ratio of their population. The required number of ultimate sampling units for the selected districts have been selected at random giving due weightage to all variables, viz. age, sex, socio-economic group, etc. Each State has

been divided into zones on the basis of geographical classification mentioned in the 1951 census. Uttar Pradesh has been divided into :

1. Himalayan Uttar Pradesh.
2. Hill and plateau division.
3. East Uttar Pradesh.
4. Central Uttar Pradesh.
5. West Uttar Pradesh.

Sample population to be taken from each of these zones is proportional to the population of the zones.

It is proposed to cover equal number of rural and urban population from a district and this number is, as far as possible, to be divided equally amongst the males and females. The age group to be covered is 0 to 20 years and in each single group it is proposed to have an equal number of males and females. The population of a district has been divided into two components of 21 each to denote the sample population from a district of males and females belonging to single age from 0 to 20 years.

Besides approaching the households, the following types of institutions would supply the subjects for the survey :

Nursery schools, basic primary schools, high schools and colleges.

For rural population, the sample has been taken from the schools located near the village and in the case of persons not going to school the co-operation of National Extension Service and Panchayat Rajya Administration has been secured for house to house survey.

The selection of subjects in a particular school would be subject to local conditions and the field staff would select, as far as possible, randomly the subjects giving due weightage to the variables mentioned above.

Out put.— The field staff eliminated personal error as indicated by the standardization report. After they have collected growth data for boys and girls, for Lucknow and Kanpur districts of Uttar Pradesh.

Part of the data has been tabulated. Table II gives the following tentative conclusions :

1. Boys exhibit a better growth than girls for stature, sitting height, circumference of wrist and weight, while the reverse is found true for circumference of calf and skinfolds of upper arm, inferior angle of scapula and superior iliac; which shows that it is not the amount of fat which influences the weight but the long bones as is indicated by the stature.
2. The rate of growth is higher for girls than for boys for all the measurements between the ages 5, 6, 7 and 8 years.

TABLE I

Showing the results of the test applied for Laboratory (L) and field work (F) done in Kurmi Pathshala, Lucknow.

MEASUREMENTS

	Stature	Sitting height	Length of arm	Biacromial breadth	Bicondylar breadth of humerus	Bicondylar breadth of femur	Facial length	Bizygomatic breadth	Depth of wrist	Circumference of wrist	Circumference of U. arm	Circumference of Chest	Circumference of C
Range of difference	L. 1.1 cm	5.1 cm	4.2 cm	2.6 cm	0.6 cm	0.8 cm	0.8 cm	0.5 cm	0.5 cm	0.5 cm	1.7 cm	...	2.8 cm
	F. 0.8 cm	0.9 cm	1.7 cm	0.6 cm	0.3 cm	0.4 cm	0.4 cm	0.2 cm	0.3 cm	0.3 cm	0.9 cm	0.5 cm	0.5 cm
Means of difference	L. 0.05217	0.05425	0.7050	0	0.08	0.005	0.025	0.09099	0.045	0.6333	0.3556	...	0.3333
	F. 0.01579	0.02	0.01667	0.01111	0.01111	0	0.1111	0.01176	0.02	0.04	0.10667	0.06	0.615
Variance of difference	L. 0.0599	0.8083	1.2508	0.347	0.0196	0.0310	0.0325	0.0082	0.0110	0.0850	0.4002	...	2.6450
	F. 0.0336	0.0431	0.1544	0.0187	0.0046	0.0088	0.0105	0.0024	0.006	0.0111	0.0621	0.0226	0.0159
't' for difference	L. 1.02	0.28	3.14**	...	2.53*	0.13	0.63	2.95**	1.70	6.53**	1.68	...	0.63
	F. 0.37	0.37	0.18	0.35	0.64	...	0.45	0.83	1.0	1.51	1.67	1.55	1.78

There has been a marked improvement from laboratory to field as the differences in the measurements have been boiled down.

TABLE II

Mean measurement in c.m. of boys (B)

Age in years	Stature		Sitting height		C I R C U M					
					Wrist		Upper Arm		Chest	
	B.	G.	B.	G.	B.	G.	B.	G.	B.	G.
4·6— 5·5	104·51	101·58	57·28	55·31	10·45	10·28	13·52	14·58	50·40	50·98
5·6— 6·5	110·47	105·95	59·49	58·01	10·85	10·56	13·76	14·63	52·45	52·61
6·6— 7·5	115·76	112·20	61·69	59·82	11·22	10·64	13·99	14·26	53·68	54·26
7·6— 8·5	116·86	116·63	62·44	61·99	11·31	11·13	14·07	15·37	54·29	54·82
8·6— 9·6	123·69	120·36	65·19	63·72	12·40	11·47	15·77	15·40	58·03	56·35
9·6— 10·5	130·54	128·00	67·57	66·10	12·50	12·02	15·70	16·27	59·96	59·24
10·6— 11·5	127·70	131·11	66·35	67·21	12·10	11·71	14·90	15·74	57·85	58·53

and girls (G) of Lucknow city according to age.

F E R E N C E				Weight lbs.		S K I N F O L D					
Hip		Calf				Upper arm		Inf. angle scapula		Superior Iliac	
B.	G.	B.	G.								
49·9	50·96	19·35	21·99	38·82	31·89	0·72	0·77	0·52	0·52	0·49	0·61
52·24	53·29	19·59	19·72	35·70	34·68	0·57	0·73	0·43	0·49	0·42	0·56
53·85	54·98	20·28	21·31	38·50	39·33	0·57	0·68	0·45	0·52	0·46	0·52
55·54	56·55	20·36	21·01	40·74	41·21	0·60	0·68	0·48	0·51	0·48	0·51
61·73	59·17	23·0	22·31	45·28	45·26	0·58	0·71	0·48	0·54	0·50	0·55
62·0	62·36	23·13	21·97	51·14	50·23	0·54	0·67	0·47	0·56	0·47	0·55
60·5	62·52	22·3	21·92	47·54	52·91	0·50	0·72	0·46	0·61	0·49	0·64

3. The rate of growth for boys is high in the ages 5 to 7 and then again from 9 to 11 years. For girls there is a retardation in growth of the circumference of calf, skinfolds of upper arm, and superior iliac.

Standardization Report.—The standardization of technique of measurements between Shri D.K. Verma and Shrimati T. Sen for the survey was done in the laboratory and in a school under field conditions. The two Anthropologists took a set of measurements on a number of individuals independently of each other and under the similar conditions. Twenty three sets of observations were selected at random from the laboratory measurements and 19 sets from the field measurements and the difference between the observations of the Anthropologists for the same individual was statistically tested on the hypothesis that there is no significant difference between the two observers. Table I gives the results of the test applied for Laboratory (L) and field work (F) done in Kurmi Pathshala, Lucknow.

A note on verification of age.—Age being an important factor in our study the correctness of school register age was tested on a random selection basis at Lucknow city.

From the field returns 50 girls were selected at random and their school age and home address was ascertained from the school. Then the parents were contacted individually and the actual age was ascertained from them. A similar procedure was followed in the case of 57 boys.

It was found that 86 per cent of the girls gave correct ages, 12 per cent of the girls gave less age and 2 per cent excess age in the school register. The mean less age for 50 girls comes to 16 days. The parents generally report the correct age in the school as they felt that the girls do not have to join services where age restrictions apply.

In the case of boys 40·35 per cent gave correct age, 47·37 per cent gave less age, and 12·28 per cent gave excess age in the school register. The mean less age for 57 boys comes to 6 months and 2 days. The parents give less age in school in those cases where they fear that their ward is likely to fail in two or more annual examinations.

12. Inquiry on relation of dietary fat and cholesterol to serum lipids under Dr. N.G. Magar at the Institute of Science, Bombay.

Animal experiments.—Forty-eight young albino rats, 4-week old and weighing between 40 g. and 50 g. have been evenly distributed into 4 groups. Each group of rats was fed for the 1st week with a fat-free diet. Afterwards they have been maintained on diets containing 20 per cent. of coco-nut, ground-nut 'Dalda' and Ghee. The composition of other ingredients was: 22 per cent casein, 4 per cent salt pure and 54 per cent cane sugar. The rats are given all the required vitamins in sufficient quantities. After the albino rats have grown up to 100 g., to 150 g. eight rats from each group are being given cholesterol in sugar daily (0·5 g. of cholesterol/kg. body-weight of rat). The remaining

4 rats are maintained on 20 per cent fat diets as controls. Rats are being weighed twice a week and their daily food intake and growth recorded.

It is proposed to sacrifice some of the rats from each group periodically and note the changes in cholesterol, phospholipids and fatty acids in various tissues along with hæmatological studies.

Poly unsaturated fatty acid.—The method for estimating poly unsaturated fatty acids was standardized and the minimum quantity of blood serum required for analysis was determined. It was found that 2.5 c.c. to 3 c.c. serum were required for good results. The poly unsaturated fatty acids obtained from blood serum were alkali-isomerized with potassium hydroxide and ethylene glycol for 15 minutes at 180°C and optical densities measured with Beckman spectrophotometer at 234, 268, 315, 346 and 376 m μ . Poly unsaturated fatty acids of blood serum from 13 normal persons have so far been estimated. Dietary fat intake of the above persons is being recorded for about seven days. It is proposed to collect data for poly unsaturated fatty acids in blood serum from different age group persons.

Phospholipids in human blood serum.—Thirty samples from males and 16 samples of females, from Bombay Municipal group of Hospitals at Sion have been analysed for total phospholipids, lecithin, sphingo-myelin, cephalin, total and free cholesterol. All these samples were from Indoor patients and their diagnosis of ailment, age and diet have been recorded.

Samples are also being analysed from normal persons. The work is in progress to collect data for all the different age-groupings.

13. Studies in vitamin A under Dr. P.D. Dalvi at the S.M.S. Medical College, Jaipur.

Beckman spectrophotometer DU Model, a gift from the Rockefeller Foundations, was repaired and adjusted according to instructions of the manufacturers. It was then calibrated in respect of wave-length scale and E values on the basis of absorbance values at different wave lengths of acidic $K_2Cr_2O_7$ and basic K_2CrO_4 solutions and also of those of vitamin A alcohol and vitamin A acetate and palmitate (the crystalline vitamin A alcohol and esters were a gift from Messrs. Hoffmann La Roché, Switzerland).

Spectroscopic solvents were prepared and those conforming to the rigid standards set for such solvents were used in the preparation of anhydro vitamin A by Dalvi's method (alumina contact process) and in chromatographic work. The methods used in the preparation of spectroscopic solvents would work cheaper than those of passing pure solvents through requisite silica jel.

Only the alumina 'O' grade (Peter Spence Co., who gave it as a gift) could effect the conversion of vitamin A concentrates (and naturally

and crystalline vitamin esters). Aluminas of other manufacture did not work.

A small quantity of anhydro vitamin A was prepared by Edisbury and Morton's method and through chromatography. The final product gave $E_{1\text{cm}}^{1\%} 368\text{m}\mu = 2200$, which will be used to study the products of interaction between and syrupy phosphoric acid, etc.

14. Inquiry on the role of nutritional deficiencies in the causation of peptic ulcer under Dr. C. Raghavachari at the Stanely Medical College, Madras.

The diet survey of 50 patients, with age varying from 25 to 55 years, has been made. Two of them were women. Excepting three patients, who come from the City the rest were from villages. Two-thirds of the patients appeared distinctly under-fed. This study has been made from the low-income group (Rs. 40/- and below). All the patients were farm labourers or coolies. Their staple food was cooked rice and ragi in the ratio of 3 : 1. All excepting two claimed to be non-vegetarians, but it is worthy of note that they took meat once or twice a month ; vegetables, such as brinjals, drum-sticks, are being used three or four times a week. Fruits were consumed infrequently. Condiments and spices were taken in large quantities. Almost all excepting three took their food at irregular intervals varying from 5 to 8 hours, and about 5 to 10 times a month they went without a meal. Milk and milk products as items of food were conspicuous by their absence. None of them took ghee but used pure vegetable oil, such as ground-nut or gingellys. Two-thirds of the patients were deficient in protein and the protein taken was mainly of vegetable origin. Fat content of the diet was very low ; the only source being from the vegetable oil used for seasoning. All of them were deficient in vitamin A. Eight of them took fish occasionally but the fish was of poor quality and the amount taken was so low that the protein and vitamin drawn from them was hardly adequate to make up the deficiencies. Ninety per cent were deficient in vitamin C and riboflavin and about half of them were deficient in vitamin P_1 and niacin. Half of them consumed black coffee at least once a day. Tobacco was used by all of them excepting in two in one form or other. Twenty per cent show angular stomatitis.

FTM analysis.—FTM tests on peptic ulcer patients are being done. No conclusions could be drawn as yet from the FTM done upto now. The meal used was cat meal conjee. It is also proposed to analyse the mucin of gastric juice in these patients. The serum total protein and serum albumin were estimated by the micro-Kjeldahl method. The serum samples were subjected to filter-paper electrophoresis using No. 1 Whatman and veronal buffer. The serum proteins separate into distinct bands of albumin and α_1 and α_2 , B and globulins ; no conclusions could be drawn from the estimations as sufficient number of analyses has not been done as yet.

The dietitian was sent for training for 15 days to the Nutritional Research Laboratories, Coonoor. Subsequently, she went round with Dr. Peter Konstam, Prof. of Surgery at Nigeria, West Africa, when he had come over to the city of Madras to investigate the causation of peptic ulcer in South India. There was also a clinical discussion with the Professor and it was found that he also concurred with us that peptic ulcers might be caused by nutritional deficiency.

15. Studies on the effect of heat on the nutritive value of edible oils under Dr. R. Rajagopalan at the Indian Institute of Science, Bangalore.

The inquiry is designed to elucidate information on the nutritive value of heated edible-vegetable oils. In nearly all parts of the world fried (in oil) materials are commonly consumed, the conditions of frying, however, will depend largely upon the local customs. In India, oils are heated in open iron pans to a fairly high temperature and the heating is continued during the actual frying of the food materials. Many of the fried products invariably contain high amounts of the oil. The duration of heating will depend upon the size of the family and the quantity of the fried food required. But, in hotels, the heating of the oil proceeds from morning till night, the oil being replenished from time to time. In the hotels, as also in the houses, the oil left over after a day's operation is taken up along with fresh oil on following or subsequent day. Under such conditions, the oxidative process is likely to be accelerated. No data could be found in the literature as to the adverse effects of consuming such oils (food materials fried in such oils).

In the preliminary trials, it was found that when ground-nut oil heated to 270°C. for 8 hours was fed to albino rats at 30 per cent level, all the rats died in a week, while the group of rats receiving the same percentage of unheated oil maintained good health. The percentage was reduced to 15. At this level, the rats lived, but their rate of growth was reduced by 50 per cent and the liver fat was increased by 100 per cent as compared with the rats receiving the unheated oil.

Confirmation of the above results was obtained by carrying out similar studies with sesame (til) and coco-nut oils.

Examination of the physical characteristics of the heated oil showed a significant reduction in iodine number. Digestibility of these oils by lipase was also reduced.

Vitamin B₁₂ did not afford any protection against the adverse symptoms produced by the heated oil. On the other hand, liver extract nearly completely eliminated the fat accumulation in the liver, though it had no effect in overcoming the growth inhibition.

It was found that a high protein diet (25 per cent casein) proved of immense benefit to the animals in as much as both the retardation in growth and accumulation of fat in the liver were eliminated. Vitamin B₁₂ in combination with a high protein diet was found to be somewhat beneficial.

Mustard oil, on heating to the same temperature and time, did not produce the adverse effects observed with the other three oils. This aspect is being studied.

16. Inquiry into the effect of insect infestation on the nutritive value of food grains under Dr. M. Swaminathan at the Central Food Technological Research Institute, Mysore.

This report covers work done in a period of two months.

OBJECT OF INVESTIGATION.

The objects of the investigation are as follows:—

- I. Development of simple methods for the assessment of the degree of infestation in foodgrains.
- II. Studies on the effect of insect infestation on the chemical composition and nutritive value of various foodgrains.

It has been reported previously from this Institute that the uric-acid content of wheat infested by insects can serve as an index of the unhygienic quality of the grain, as uric acid is an important constituent in the excreta of insects. Taking this as the basis, it was considered desirable to find out if there is any correlation between the uric-acid content, insect numbers and kernel damage in different grains infested by various insects.

The following insects were readily available and assessment of damage caused by them to the respective grains was taken up :

1. *Calandra oryzae* on whole jowar.
2. *Tribolium castaneum* on crushed jowar.
3. *Rhizopertha dominica* on crushed jowar.
4. *Oryzaephilus surinamensis* on whole rice.

Note :—Crushed jowar was used in case of insects No. 2 and 3 as they are secondary pests of stored grains.

EXPERIMENTAL PROCEDURE.

The insects were collected in large numbers from the stock culture and conditioned to the required grain for 48 hours at a temperature of 28°C and a relative humidity of 70 per cent. They were then starved for 24 hours and 100 insects were let into flasks containing 10 of the respective grains. The insects were allowed to infest the grains for 96 hours at the end of which they were removed. The number of insects dead and alive after this period was noted. The grain samples were then fumigated with ethylene dibromide, the dose being 3 lb./1,000 c.ft. to prevent any development of eggs. A control was kept for every sample of infested material.

All the samples, after fumigation, were ground to a fine powder and the uric-acid contents of these were estimated by the following procedure : A sample of the flour (2.5) was weighed into a flask. One hundred c.c. of water and 1 cc. of CHCl_3 were added. The mixture was shaken for a few minutes and left overnight. It was then mixed well in a Waring blender for 3 minutes, centrifuged and decanted. To 50 c.c. of the centrifugate, were added 5 c.c. of 10 per cent sodium tungstate and 5 c.c. of $\frac{2}{3} \text{N}_2\text{H SO}_4$ to precipitate the proteins. The mixture was allowed to stand for 15 minutes and filtered. Twenty c.c. of the clear filtrate (equivalent to 1/6th the quantity of flour initially taken) were used for determining the uric acid present according to the method of Benedict and Franke (*Jour. Biol. Chem.*, **52**, 387, (1922)). The value for the 'apparent' uric acid contained in the food material as determined in the control samples was subtracted from the values obtained for the experimental samples.

RESULTS.

The average values of uric acid contributed by the adult stages of some of the insects infesting jowar for a period of four days are given below :—

- (1) *Tribolium castaneum* (100 insects)—0.71 mg. of uric acid/10g. of crushed jowar.
- (2) *Calandra oryzae* (103 insects)—0.31 mg. of uric acid/10g. of whole jowar.
- (3) *Rhizopertha dominica* (100 insects)—0.088 mg. of uric acid/10 g. of crushed jowar.

It will be seen that the uric-acid content of whole jowar infested with *Calandra* is considerably less, and that of crushed jowar infested with *Rhizopertha* still less, when compared to that of crushed jowar infested with *Tribolium*. The quantity of uric acid excreted appears to be related to the size of the insect. Further experiments are in progress.

The contribution of uric acid by the larval stages of the insects and the effect of insect infestation on the chemical composition of foodgrains are under study.

MENTAL HEALTH

1. Inquiry to study the biochemical and hæmatological data associated with mental disorders under Dr. M.V. Govindaswamy at the All-India Institute of Mental Health, Bangalore.

The above inquiry was undertaken : (i) To collect basic hæmatological and biochemical data in mental patients in India and their

corresponding controls, (ii) a comparison of the values for the patients and the controls, (iii) the values for the patients belonging to different types of mental disorder, and (iv) whether there were variations in the same patient, of prognostic importance, during his clinical course, are other objects of the inquiry.

The inquiry was commenced in May after preliminary standardization of the techniques, etc. Patients admitted to the Mental Hospital, Bangalore, were the subjects of study with their siblings as controls. In order to avoid errors in the selection of patients 'random selection technique' was employed. Based on the previous year's admissions and the distribution of sex proportion, sixty random numbers were selected in proportion of female 3:2 (to cover the period of five months, *i. e.* from May to the end of September). The relevant socio-economic data of the patients and the controls were also obtained.

Hæmatological picture, which included red blood corpuscle count (r.b.c.), white blood corpuscle count (w.b.c.), hæmoglobin (Hb), erythrocyte sedimentation rate (e.s.c.), packed cell volume (p.c.v.), specific gravity (SpG), was obtained in the subjects for investigation. The biochemical investigations in blood included the estimations of Glucose in blood, total non-protein nitrogen (N.P.N.) in blood, total proteins and A/G ratio in serum, sodium and chlorides in plasma and serum potassium. Any condition which upsets the 'homostatic mechanism' has its influence on the mental state of an individual, and hence the importance was of these investigations. Routine urinary examination was also carried out. The data obtained and presented have been subjected to statistical analysis.

Apart from these investigations on random samples (36 male patients *plus* 24 female patients), similar studies were conducted on other patients (25 male patients and 15 female patients) wherever suitable controls were available. Routine investigations of the cerebrospinal fluid (C.S.F.) were conducted in some cases. Blood sugar estimations were carried out in patients undergoing insulin shock treatment and glucose tolerance tests throughout the period of the treatment.

The following points arise out of these preliminary investigations : (i) the investigations should be extended to a larger number of cases representing 1,500 patients with controls, (ii) additional techniques of study are necessary. Electrophoretic study of the 'protein profile' has been started with special reference to types of treatment and clinical course in the various mental disorders, (iii) it is also proposed to commence investigations on the basal metabolic rate (B.M.R.), and spectrophotometric studies for which equipment has been received, and (iv) after necessary preliminary studies, attention will be concentrated on special types of mental disorders including epilepsy and at different age groups (specially children and people above 50).

PHYSIOLOGY AND PHARMACOLOGY

1. Indigenous drugs inquiry under Dr. M.L. Chatterjee at the School of Tropical Medicine, Calcutta.

Total alkaloids of *Rawolfia serpentina* Benth have previously been shown to possess anti-adrenaline, anti-acetylcholine, anti-histamine and anti-barium properties. As *Rawolfia serpentina* (R.S.) contains more than twenty alkaloids, it was considered worthwhile to isolate different fractions by the technique of counter-current distribution in a state of relative purity, identify the alkaloids whenever possible, and undertake a systematic screening of general pharmacological properties of the isolated components in intact animals as well as on isolated tissues with special emphasis on their potentiating or antagonizing effect against neuro-transmitter agents, e.g., adrenaline, acetylcholine and histamine. Besides elucidating the mode of action of *Rauwolfia* alkaloids and the mechanism of essential hypertension in general, the present observations, when completed may explain the relative advantages of R.S. (total) over reserpine alone in some of their pharmacological properties. A proper understanding of the effects and the mechanism of action of individual alkaloids will enable the clinicians and the pharmacutists to so modify and reconstitute the *Rauwolfia* drug that it may combine the advantages of both reserpine and R.S. (total). Moreover, the anti-histaminic and other antagonistic actions exhibited by some of the fractions, as evident from the present investigation, may find some clinical use hitherto unexplored, either when used alone or in suitable combination.

Pharmacological investigations with isolated fractions of Rauwolfia serpentina.—The following observations emerge from systematic pharmacological investigations into the effect of different fractions of R.S. (Benth) obtained by counter-current distribution of 2 per cent hydrochloric-acid extract of total soluble alkaloids from R.S. mentioned hereunder as fractions B₁, B₂, etc.

(i) *Fraction B₁*, identified as ajmaline, causes a transient fall of blood pressure, stimulates respiration and possesses slight anti-histamine action on blood pressure, respiration and on isolated guinea-pig-ileum. The experiment was corroborated by similar study with an authentic sample of ajmaline (Ricker Lab. California, U.S.A.)

(ii) *Fraction B₂*, which contains a number of alkaloids not fractionated as yet, causes depression of blood pressure, rate of respiration and contraction of the nictitating membrane even after atropinization. This fraction partly antagonizes the effect of adrenaline and the nicotinic action of acetylcholine on blood pressure and nictitating membrane. It possesses anti-histaminic action on denervated nictitating membrane but not on blood pressure.

(iii) *Fraction B₃*, which consists of a fluorescent alkaloid not identified so far together with traces of ajmaline or its isomer, causes a mild rise of blood pressure and contraction of denervated nictitating membrane. It potentiates the action of adrenaline, nor-adrenaline and histamine on denervated nictitating membrane. The effects of these neuro-transmitter agents remain unaltered on blood pressure.

(iv) *Fraction B₆*, identified as serpentine, causes a fall of blood pressure. It possesses antihistamine action on blood pressure and on isolated guinea-pig ileum. The drug improves the amplitude of contraction and shows anti-adrenaline and anti-acetylcholine actions on isolated rabbit auricles.

(v) *Fractions B₇ and B₈*, comprising of serpine and another unidentified alkaloid, cause fall of blood pressure and marked depression of respiration. The drug exhibits delayed antagonism against adrenaline, nor-adrenaline and nicotinic action of acetylcholine on blood pressure. It shows anti-histamine action on blood pressure, respiration and on Isolated guinea-pig ileum.

(vi) *Fractions B₄ and B₅*.—Investigation on the pharmacological properties of these fractions is in progress.

Action of R.S. (total) on amphibian heart.—The action of adrenaline on perfused toad heart remains unaltered or potentiated or diminished, depending on the concentration of R.S. (total) from lower to higher ranges used immediately before the injection of adrenaline. In case of continuous perfusion, R.S. facilitates the action of adrenaline, though by itself it causes a good deal of depression.

Histamine content of skin lesions of leprosy.—Biological tests, using isolated guinea-pig ileum, show that the histamine content of tuberculoïd lesions of leprosy far exceeds that of the corresponding normal skin of the opposite side. In neuro-macular lesions, the histamine content is similar to that of the normal skin of the correspondingly opposite site. The above work was carried out in collaboration with the Department of Leprosy, School of Tropical Medicine, Calcutta.

Chemical investigations with Rauvolfia serpentina.—With the help of a 80-tube semi-continuous counter current distribution apparatus set up under the direct supervision of the inquiry, it has been possible to resolve the total soluble alkaloids into eight different fractions. Of them, three have been identified as ajmaline, serpentine and serpine by means of paper chromatography (both one dimensional and circular), absorption spectrophotometry and parallel distribution studies with pure and authentic samples of the three alkaloids. Pharmacological tests lend further support as to the purity of the characterized fractions. Relative percentages of the different fractions have been calculated on the basis of distribution patterns obtained by gravimetric analysis.

The technique of paper chromatography has been employed as an analytical tool as a means of check of purity of the different fractions. It has been shown that all humps obtained on the distribution patterns do not necessarily signify the homogeneous nature of the components. Furthermore, partition mechanism, with water alone as the developing solvent in an anionic or cationic atmosphere, calls for fresh consideration.

Earlier, it has been reported that the distribution of fluorescence does not always run parallel with the mass of the components. It has now been concluded that total fluorescence of R.S. is not connected with principal alkaloidal mass,

Prior to fractionation proper, the alcoholic percolate has been pretreated in order to remove the associated impurities which otherwise would have appeared as contaminants in the isolated fractions. Moreover, such impurities often carry over traces of other alkaloids as trailing agents into the region of an pure component.

Evidence has been presented that the technique of counter-current distribution alone can be successfully utilized for the resolution of *Rauvolfia* alkaloids, provided adequate care is taken with regard to the number of transfers and proper selection of solvents. The technique developed in this Laboratory may be employed for the isolation of alkaloids from species of *Rauvolfia* other than *Rauvolfia serpentina* Benth.

Isolation of an antibacterial substance from Drynaria quercifolia.—The antibacterial principle from a local pteridophyte *Drynaria quercifolia* has been isolated in crude form. The substance, in its present state of purity, has been found to possess activity against *Staphylococcus aureus* and *Mycobacterium*.

Metabolic and clinical studies with reserpine and R.S. (total).—Preliminary study on the excretion pattern of reserpine in human urine by means of solvent extraction and paper chromatography has shown that four fluorescent metabolites appear on the chromatogram in addition to a substance showing bluish fluorescence. Similar study with R.S. (total) indicates the presence of a large number of components in the metabolite which appear as overlapping zones on the paper chromatogram. Further investigation on the excretion pattern of the drugs by means of the technique of counter-current distribution has been undertaken with a large volume of urine as the starting material in order to isolate the metabolites, both fluorescent and non-fluorescent.

Clinical trials with R.S. (total), reserpine and reserpine-free total alkaloids have been undertaken on patients suffering from essential hypertension in Carmichael Hospital for Tropical Diseases, School of Tropical Medicine, Calcutta. Further trial is necessary before any statistical significance can be attached to the results obtained.

2. Indigenous drugs inquiry under Bt. Col. R.N. Chopra at the Drug Research Laboratory, Jammu, Tawi.

Pharmacological screening of indigenous medicinal plants was continued and the following five plants were investigated :—

1. *Achyranthes aspera* (vern. Apamarg).—It is found throughout India and commonly used as stomachic, carminative and emetic and useful in bronchitis, heart diseases, piles, dyspepsia and dysentery. The alcoholic extract of the whole plant produces relaxation of the smooth muscle of intestine and contraction of uterine musculature. The heart is moderately depressed and blood vessels constricted. Both the frequency and amplitude of respiratory movements are transitorily increased,

2. *Cleome viscosa* (vern. Hurhur).—The plant is found throughout the tropical parts of India and used as stomachic, diuretic and anthelmintic. It is commonly prescribed for the treatment of skin diseases, ulcers, leprosy, fevers and uterine troubles. The alcoholic extract of the plant produces a transient fall of blood pressure, which soon recovers. Blood vessels are not effected. Respiratory movements are stimulated, both amplitude as well as rate, are increased. It relaxes smooth muscle of intestine and stimulates uterus.

3. *Sphaeranthus indicus* (vern. Gorakmundi).—The herb is found throughout India and Ceylon and commonly used by indigenous practitioners as tonic, alterative and anthelmintic and prescribed for the treatment of insanity, tuberculous glands, asthma, leucoderma, dysentery and anæmia. The extract of the whole plant produces transient depression of blood pressure and constriction of splanchnic blood vessels. Respiratory system is not much affected. It produces a well-marked relaxation of smooth muscle of intant and isolated intestine. Guinea-pig uterus is estimulated.

4. *Anagallis arvensis* (vern. Jonkmari).—It is found more or less throughout India and used for the treatment of inflammations, sores, opthalmia, cerebral affections leprosy, epilepsy and mania. Pharmacological investigations show that it stimulates smooth muscle of intestine and uterus in very small doses. The heart is depressed and respiration temporarily stimulated. The plant appears to be promising and further work will be taken up.

5. *Datisca cannabina* (vern. Akalbir).—It is a glabrous herb and found in temperate and sub-tropical Western Himalayas from Kashmir to Nepal. The plant is bitter and purgative and commonly prescribed as an expectorant, diuretic and febrifuge. The root is used as a sedative in rheumatism. The action of the extract on cardiovascular and respiratory system is not well marked. The smooth muscle of intestine and uterus are relaxed. This has been observed even after induced myogenic and neurogenic spasms.

II. *Screening of anti-bacterial properties of indigenous plants.*—The screening of indigenous plant alleged to possess anti-bacterial properties was continued this year and the alcoholic extracts of the following plants tested *in vitro* against the representatives of acid-fast and non-acid fast pathogens :—

1. <i>Artabotrys odoratissimus</i>	(Vern. Kandhar)
2. <i>Citrullus colocynthis</i> .	
3. <i>Momordica charantia</i>	(Vern. Karela)
4. <i>Argemone maxicana</i>	(Vern. Pila Dhatura)
5. <i>Alpinia galanga</i>	(Vern. Kulanjan)
6. <i>Asparagus racemosus</i>	(Vern. Stawar)
7. <i>Lactuca scariola</i>	(Vern. Kahoo)
8. <i>Hypericum perforatum</i> .	(Tern. Bassant)
9. <i>Vebascum thapsus</i>	(Vern. Gidar Tamaku)
10. <i>Ageratum conyzoides</i>	(Vern. Sahadevi)

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|--------------------------------|----------------------|
| 11. <i>Agavae americana</i> | (Vern. Bankaara) |
| 12. <i>Tribulus terrestris</i> | (Vern. Chota Gokhru) |
| 13. <i>Sedum asiaticum</i> . | |
| 14. <i>Cedrus deodara</i> | (Vern. Deodar) |
| 15. <i>Acorous calamus</i> | (Vern. Bach) |
| 15. <i>Phaseolus trilobus</i> | (Vern. Moth) |

The alcoholic extract from the roots of *Alpinia galanga* and *Acorous calamus* showed definite anti-microbial activity.

III. *In vitro* study of tuberculostatic properties of indigenous drugs.—The anti-bacterial properties of the volatile principles from the rhizomes of *Alpinia galanga* and *Acorous calamus* have been studied. Both the oils show marked anti-tubercular action in *in vitro* studies. The oil from the roots of *Acorous calamus* inhibits the growth of *M. tuberculosis* in a concentration of 10 mcg/c.c. (1 in 100,000) and oil from the roots *Alpinia galanga* inhibits the growth in a concentration of 25 mcg/c.c. (1 in 40,000). Both the oils inhibit the growth of Gram-negative organisms in concentrations ranging from 0.4 mg./c.c. to 0.6 mg./c.c. (1 in 2,500 to 1 in 1,666). The oil has no inhibitory effect on the growth of Gram-positive organisms, of which *M. Pyogenes* var. *aureus* has been taken as typical representatives, even in a concentration of 1 mg./c.c. (1 in 1,000).

Acute and chronic toxicity studies on the oils were carried out in guinea-pigs by intraperitoneal route. The LD 50 and 19/20 confidence limits of the oils of *Alpinia galanga* and *Acorous calamus* as calculated by Litchi field and Wilcoxon method are 0.068 (0.056 to 0.076) c.c. and 0.0275 (0.0214 to 0.0354) c.c. per 100 g. body-weight, respectively. In chronic toxicity studies the oils of *Alpinia galanga* and *Acorous calamus*, when administered intraperitoneally in doses of 0.02 c.c. and 0.01 c.c. per 100 g. body-weight, respectively, for six days a week for six weeks did not produce any gross pathological changes in the internal organs of the animals.

3. Investigation into the study of cortical hormones under Dr. B. Mukerjee at the Central Drug Research Institute, Lucknow.

Testosterone propionate, administered to young male rats at a relatively high dose level caused stimulation of the adrenocortical activity along with fatty metaplasia of the cortical cells. Such stimulation is due neither to the alleged protein anabolic action/potassium retaining property nor to the non-specific damaging action of testosterone. The direct influence of the sex hormone, if any, on the adrenal cortex is solely insufficient to account for it. It is, therefore, presumed that testosterone at the above dose level provokes enhanced output of pituitary ACTH and thus causes increased adrenocortical activity. Thyroid gland does not seem to mediate in such a process.

Contrary to current belief, chronic overdosage with testosterone propionate was noted to produce adrenocortical stimulation along with pronounced fatty metaplasia of the cortical cells,

Contradictory reports about the behaviour of adrenal cortex under the influence of testosterone have appeared at different times. Basing on the results of our investigation it is emphasized that such differential response is probably related to the different dose levels of the sex hormones employed by different investigators and also to different duration of hormone treatment. A tentative hypothesis has been put forward in which it is pointed out that testosterone exerts its influence on the adrenal cortex through several pathways. It is probably the dosage level of the hormone which determines through which pathway the androgen action is predominantly exerted at any state. The response of the adrenal cortex is primarily conditioned by the intensity and nature of androgen action.

4. Inquiry on biogenesis of alkaloids in plants under Dr. B.C. Bose at the M.G.M. Medical College, Indore.

Effect of potassium nitrate and the enzyme preparations on nicotine synthesis in the leaves of N. tabacum.—In continuation of the work on the *in vitro* synthesis of nicotine in the tissues of *N. tabacum* plants, further investigations were taken up by studying the role played by the enzyme preparations from the leaf, root and stem of *N. tabacum* and *N. glauca* in the synthesis of nicotine. Leaves of *N. tabacum*, cut into thin slices, were incubated with the salt solution, phosphate buffer, the enzyme preparations and different doses of potassium nitrate. In case of the control sets the enzyme preparation was replaced by distilled water. After the completion of 24-hour incubation at 37°C, the alkaloids were collected by steam distillation and estimated by the aniline and cyanogen bromide-colorimetric method. Increase in the nicotine content of the leaves due to potassium nitrate was found but no further enhancement of synthesis was observed due to the addition of enzyme preparation.

For further investigating the effect of potassium nitrate and ammonium sulphate, on the enzyme preparations, various doses of these inorganic nitrogenous sources were incubated in the salt solution and phosphate buffer at pH 7, with the enzyme extract from the different tissues of *N. tabacum* and *N. glauca*. Estimation, after 24-hour incubation at 37°C did not show any alkaloid formation, indicating the absence of any effect being exercised by the above compounds on the isolated enzyme preparations.

Studies on the enzymes in the conversion of tryptophane and other amino-acid precursors to alkaloids of the pyridine group.—The enzyme extract from the different tissues of the two tobacco plants were prepared and incubated as before with different doses of tryptophane, ornithine, proline, arginine and anthranilic acid, respectively. On analysing, after completion of the incubation, no conversion of these amino acids to the pyridine alkaloids was observed. In order to find out whether the presence of leaf slices could activate the enzyme preparations, another series of experiment was done. Slices of *N. tabacum* leaves with dose of tryptophane, kept as control set, were incubated with the enzyme preparations. The effect of vitamins,

riboflavin and nicotinic acid with the enzyme preparations, was also studied¹. Nicotine formation was not found to be effected by the enzyme preparations in these experiments.

This inability of the isolated enzyme preparations, from the tissues of the tobacco plants, in the conversion of amino-acid precursors to alkaloids of the pyridine group shows that the presence of particular co-enzymes is also essential in the alkaloid formation. This is in conformity with the view that the primary control in the alkaloid formation is probably governed by 'gene complex' and single gene may directly control the existence or operation of the individual enzyme. Any new enzyme, stable only in the presence of its substrate, may be formed due to the mutation of a gene. Such enzymes are an essential factor in the conversion of amino-acid precursors to alkaloids in the plants. The isolated enzyme preparations, lacking the above coenzymes may thus be ineffective in alkaloid formation.

5. Study on the anti-veratrinic antiaccelerator and anti-arrhythmic activity of indigenous and synthetic drugs and to determine the action of anæsthetics and pre-anæsthetics on a cardiac automaticity and conductivity under Dr. R. B. Arora at the S.M.S. Medical College, Jaipur.

I. *Three new methods have been established.*—(i) The ability of a drug to antagonize the veratrine response produced in skeletal muscle of frog is a true guide to its anti-arrhythmic activity. It has therefore, been employed as a screening procedure for testing the quinidine like activity of drugs. It was superior to Dawe's isolated rabbit-auricle's technique, which is based on the assumption that prolongation of refractory period is a measure of antifibrillatory potency of the drug. This view has now met with scepticism since drugs, such as emetine, procaine and rauwolfia alkaloids, have no effect on refractory period but exert antiarrhythmic action. These compounds have, however, antiveratrinic properties and hence employment of antiveratrinic method for screening the drugs for their antiarrhythmic actions is favoured. (ii) A 'modified closed-chest' technique for the production and maintenance of A-V nodal rhythm in dogs has been established and it is better suited to the tropical conditions. (iii) A procedure has been adopted to test the antagonism to veratrine response of a compound as its specific action and not merely a manifestation of its toxicity.

II. Anti-arrhythmic activity of anti-malarials (plaquenil, isopentaquin, daraprim, paludrine, and supazine), local anæsthetics (pyrrollocaine, U-0045 lactate, U-0069 lactate, thenfadil HCl, *Rauwolfia serpentina* alkaloids (ajmaline, serpentine, reserpeine, rescinnamine and rauwiloid) and indigenous Indian drugs (essential oil of *Acorus calamus* and fixed oil of *Nardostachys jatamansi* have been tested by employing a variety of tests in different species of laboratory animals. Of these, plaquenil, ajmaline and fixed oil of *N. jatamansi* show promise of therapeutic usefulness in selected cases of cardiac rhythms in view of their effectiveness in experimental arrhythmias and relatively low toxicity.

III. Coumarin derivatives, cinchona alkaloids, synthetic antimalarials, cupreines, *Rauwolfia serpentina* alkaloids and some recently synthesized local anæsthetics have been shown to exert a specific action in antagonising the veratrine response produced in skeletal muscles of the frog by 1:10 million veratridine.

IV. *Vasicine*.—A new water-soluble alkaloid from the leaves of *Adhatoda Vasaka*, has been investigated for its respiratory stimulant activity, both in unanæsthetized and morphinized albino rabbits. It has respiratory stimulant property comparable to that of cardiazol. In view of its very low toxicity, this property of the herbal preparation may be put to clinical use.

V. *The actions of 21-hydroxypregnanedione sodium succinate (hydroxydione, Viadril)*, a water-soluble steroid anæsthetic, has been investigated on the cardio-vascular system and respiration of cats and dogs normal and hypodynamic amphibian hearts, rabbit's and guinea-pig's ileum, and tracheal muscles of cat, dog, rabbit and guinea-pig. In cats and dogs hydroxydione produced an initial transitory hypotension due to vasodilation followed by rise above initial level due to vasoconstriction and lastly, in doses above 40 mg./kg., a gradual hypotension due to depression of vasomotor centre. It had insignificant effect on cardiac rate and none on rhythm. In amphibian hearts, it had negative chronotropic and in low concentrations positive and inotropic action in normal and hypodynamic hearts. It delayed conduction and caused irregularities. In cats and dogs it had no significant effect on e.c.g. pattern, P-R and R-T intervals, but in amphibian hearts it broadened T wave and increased P-R and R-T intervals. Hydroxydione in four times the dose of thiopental depressed the heart much less than the latter, in intact and heart-lung preparations of dogs. It depressed respiration much less than thiopental.

Hydroxydione had pronounced relaxant effect in rabbit's and guinea-pig's ileum and antispasmodic action against acetylcholine and potassium chloride induced spasm in tracheal and ileal muscles, of cats, dogs, guinea-pigs and rabbits.

VI. *Jatamansi*.—Experiments with essential oil of *N. jatamansi* in dogs showed that it has prolonged and pronounced hypotensive effect lasting for several hours. The mechanism of this hypotension was investigated by experimenting on intact and spinal dogs and cats, heart-lung preparation of dogs, dogs hind limb perfusion and on isolated frog's heart. In doses of 0.1 to 1.0 mg./kg., it caused moderate or marked fall in blood pressure lasting for 2 to 6 hours or more. It showed tachyphylaxis. Intracisternal administration and spinal animal experiments showed that it does not depress vasomotor centre. It blocked proprioceptive blood pressure regulating reflexes. In dog's and frog's hearts it had slight negative inotropic and positive chronotropic effects. In moderate doses it had no effect on e.c.g. pattern. It did not block autonomic ganglionic transmission but had some adrenolytic action. It caused stimulation of respiration more in rate and sometimes in volume. In dogs hind limb perfusion experiments, the oil in 1:100,000 concentration caused slight vasodilatation, while in 1:10,000 concentration, it

increased blood flow by 50 per cent. In four heart-lung preparations of dogs, *N. jatamansi* essential oil had slight degree of cardio-depressant effect. It was confirmed by 'competence test' of Krayner *et al* (1937).

The drug appears to cause hypotension by peripheral vasodilator action, while negative inotropic depression of the proprioceptive blood pressure regulating reflexes and adrenolytic actions are contributory factors. The drug may possibly be used in cases of hypertension.

6. Inquiry into the antidiabetic properties of some selected indigenous plants under Dr. (Smt.) Ranita Aiman at the B.J. Medical College, Poona.

- (i) *Pterocarpus Marsupium*.
- (ii) *Bassia Longifolia*.
- (iii) *Casaria Esculenta*.

Studies were undertaken to determine if these drugs exert any action on the blood sugar of normal or animals made diabetic by alloxan or pancreatectomy. Animals used were rabbits and dogs.

(i) *Pterocarpus Marsupium* was studied on

(A) The Fasting Blood Sugar (F.B.S.) of normal rabbits. Three controlled estimations were done as follows on five rabbits.

- (a) Every $\frac{1}{2}$, 1, 2, 3, and 4 hrs. without dosage of any kind.
- (b) Before insertion of stomach tube and thereafter as above.
- (c) Before administration of 50 c.c. of distilled water by stomach tube and thereafter as in *a*.
- (d) A cold water extract of the drug was then given by a stomach tube and F.B.S. estimations done as in *c*.
- (e) The above was repeated using double the dosage.
- (f) A similar study as outlined in *a*. to *d*. was done on three rabbits with a hot water extract.

The results were plotted. The curves with both dosages of cold water extract were within the limits of the control curves. The curves with hot water extract gave lower values for the 2nd, 3rd and 4th hrs. samples when the averages for all three rabbits were calculated and compared with the corresponding controls.

(B) Alloxam Diabetic Rabbits : Five normal rabbits were given the cold water extract daily by stomach tube for varying periods of time and two hours before diabetogenic dose of alloxan monohydrate (200 mg/kg. body wt.) intravenously. Blood sugar studies were done before and periodically at $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4, 6, 8, 24, 48, 72 hrs. after alloxan. The Drug was continued during the post-alloxan period.

Four rabbits used as control were given alloxan and investigated as above without drug administration.

The data thus obtained, failed to reveal any marked change in the blood sugar values of the drug treated group.

(C) Normal Dogs : F.B.S. estimations and dextrose tolerance curves were done before and during a period of prolonged administration of the drug (6 to 12 months) given in the meals to 5 dogs.

The drug appeared to have no effect on either F.B.S. or dextrose tolerance of normal dogs.

(D) Alloxan Diabetic Dogs : Three dogs were given the drug for a prolonged period and studied as above. They were then given intravenous alloxan monohydrate (60 mg./kg. b.w.) and blood sugar estimations carried out as described in B. Dextrose tolerance curves were done on the 12 and 30th day and F.B.S. estimation on the 24th day of alloxan administration, drug being administered throughout.

Two dogs used as control were given alloxan as above, without drug administration.

On comparing the results it would appear that the blood sugar values of the drug treated group are less in both the initial and final hyperglycaemic and secondary hypoglycaemic phases, and higher in the initial hypoglycaemic phase.

(E) Depancreatized Dogs : The drug was given to two dogs for ten days in their food during which time three F.B.S. estimations were done. They were then subjected to operation. One animal died a few hours after, but showed hyperglycaemia before death. The other has been under observation for 12 days, the drug being continued from the 3rd post-operative day. Thus far it has shown no evidence of diabetes. The urine showed a trace of sugar on the first day of operation. This dog is still under study.

(ii) *Bassia Longifolia* : The studies of last year are being continued.

Alloxan Diabetic Rabbits : 4 Rabbits are being studied as described in i. B. Two have been given alloxan while the other two are yet to be studied.

(iii) *Casaria Esculenta* : The studies of the last year were continued.

Depancreatized Dog : As described in i. E. The drug was given to a dog for 15 days and studied for 20 days after pancreatectomy. Diabetes was intense and insulin had to be used to partially control it, in spite of which it died on the 20th day. Postmortem and histological examination revealed broncho-pneumonia and nutritional atrophy of the liver.

7. Inquiry on the study of anthelmintic action of shell oil of cashewnut under Dr. N.V. Bhaduri, at the School of Tropical Medicine, Calcutta.

Leao and Eichbaum (1947) found that the oil extracted from the shells of cashewnuts had a fair degree of anthelmintic action against the hookworms and roundworms of dogs. Eichbaum *et al* (1950) used this extract on small number of human beings infected with hookworms. *Ascaris lumbricoides* and *Trichuris trichiura* and obtained encouraging results. They did not, however, state the method of preparation of the oil. Bhaduri and Sanyal (1952) used the oil which one of them (P.K.S.) prepared from the shells of cashewnuts, on human beings and reported, in a preliminary note, that the prepared oil exhibited good anthelmintic properties against hookworms, *Ascaris lumbricoides* and *Fasciolopsis buski* and was a non-toxic and well-tolerated remedy.

Further study on the whole extract (Bhaduri *et al* 1953, 1954) confirmed the preliminary observations of Bhaduri and Sanyal and showed that the whole extract was of value for the treatment of infection by *Hymenolepis nana*, and *H. diminuta* and its action against *Enterobius vermicularis* and *Strongyloides stercoralis* was also promising. No action was observed against *Tænia saginata*, *T. solium* and *Trichuris trichiura*.

The total extract was irritant to skin and mucous membranes and, therefore, it had to be put in hard gelatin capsules to be administered to patients. When the extract was mixed with milk and given, the anthelmintic action was much lowered.

Systematic chemical investigations of the whole extract showed that the oily extract is not a true oil but is composed of anacardic acid (2-hydroxy 6-n-pentadecadienyl benzoic acid) and cardol (5 pentadecadienyl resorcinol) and cardanol (3-pentadecadienyl phenol). If the extract is, however, obtained by roasting of the shells, the anacardic acid mostly decomposes into cardanol. Study on separation of these fractions was next done and a method was devised to obtain cardol in pure form suitable for use on human beings.

The cardol fraction, a thick brown liquid, was next taken up for study of its anthelmintic properties. Bhaduri *et al* (1954) found this fraction to be a non-toxic and well-tolerated remedy, effective against *Ascaris lumbricoides* and also with some action against *Hymenolepis nana*, *H. diminuta* and *Enterobius vermicularis*. There appeared to be very few contra-indications for use of this drug which could be used even on pregnant mothers with safety.

For the study of the cardanol fraction a method was devised to obtain it in pure form from the anacardic acid. This fraction was also a thick brown irritant liquid. It was found by Bhaduri *et al* (1956) to have some degree anthelmintic action against the round worm and hookworms of man. This fraction too was found to be a nontoxic substance, well tolerated by the patients.

Thus, the total shell extract of cashewnuts and its constituents were all found to be a good, nontoxic, well tolerated indigenous remedy

against the common intestinal worms of man. The liquid form of the drug stood on the way of its wide use and not very suitable for children. The most advantageous point was the fact that no contraindications were observed to the use of this indigenous anthelmintic with a broad range of action.

The cardol fraction has very recently been converted into a solid light power by hydrogenation. The form offers better standardization, increased dosage and can be very conveniently used on patients. Study of its anthelmintic properties has just begun and the results on 2 cases appear to be good and it is expected that this drug will be of immense value to India.

8. A study of the modification by quinine of the hæmolytic response of erythrocytes to various hæmolytic agents under Dr. R. Singh Grewal at the Medical College, Nagpur.

It was observed that quinine in 1 in 10,000 dilution rendered the erythrocytes more prone to hæmolysis by Saponin in vitro. The degree of hæmolysis was estimated by photoelectric colorimetric method. The method is based on the principle of estimating the total hæmoglobin content of that particular sample of blood and then the amount of hæmoglobin in the supernatant after producing hæmolysis by the addition of hæmolytic agent. The degree of hæmolysis is expressed in terms of total hæmoglobin content which represents 100% hæmolysis.

More experiments were done to confirm the original observation that quinine in 1 in 10,000 dilution makes the cells more susceptible to the action of Saponin. To set up an experiment dilutions of Saponin ranging, from 1-15000 to 1-40,000 in Simmel's solution were taken. To this Quinine dihydrochloride was added so as have a final dilution of 1 in 10,000 and then 0.1 cc. of heparinized blood was added. The tubes were kept at room temperature for half an hour and centrifuged for 15 minutes. The hæmoglobin content was estimated by Photoelectric colorimetric method and the degree of hæmolysis calculated.

The degree of hæmolysis produced by the same concentration of saponin is more when quinine 1 in 10,000 dilution is added to the hæmolytic system. Thus quinine renders the cells more susceptible to the action of Saponin.

Experiments were done in rabbits to find out whether quinine produces the same effect in vivo. First the degree of hæmolysis produced by varying dilutions of Saponin was tested. Then 65 mg. Quinine dihydrochlor in 20 cc. of 5% glucose Saline was slowly injected intravenously. Then the degree of hæmolysis produced by same dilutions of Saponin was tested 1/2 hour and one and half hours after the injection.

In 2 rabbits the degree of hæmolysis produced by Saponin was not altered after injection of 65 mg. of Quinine. It appears that quinine when given intravenously can increase the degree of hæmolysis produced by Saponin.

9. Inquiry on the action of *Herpestis monniera* bacopa herb (Brahmi) on the nervous system under Dr. C.L. Malhotra at the Lady Hardinge Medical College, New Delhi.

The previous work on pharmacology of *Herpestis monniera* carried out in this laboratory from 1st April, 1955 to 30th September 1955 indicated that the dried alcoholic extract of *Herpestis monniera* had the following effects :—

- (a) Depression of the nervous system in frogs.
- (b) Neuro-muscular blocking effect on nerve muscle preparation of frog.
- (c) Direct stimulation of frog's heart-cardiotonic (both in isolated as well as intact heart).
- (d) Vaso-constriction of blood vessels in frogs.

Further work on dried alcoholic extract was carried out in rats. A dose of 200 mg./100 g. body-weight was lethal to all the rats tested. There was marked neurological deficit as shown by positional sense righting, gait and stance, muscle tone and equilibration tests. This was followed by loss of all voluntary movements, marked respiratory depression and death within 45 minutes. With dose of 150 mg./100 g. body-weight, same effects were noted but survival time was prolonged to about two hours. LD₅₀, in rats, varied from 30 to 40 mg./100 g. body-weight, both when given orally as well as when injected intraperitoneally. With doses varying from 10 to 20 mg./100 g. body-weight there was neurological deficit of varying degrees as determined by various tests mentioned above.

After working out the preliminary pharmacology of the dried alcoholic extract, different parts of the plant (stems, leaves, etc.) were extracted successively and separately with rectified spirit, absolute alcohol, petroleum ether and chloroform in hot and cold. The stems and leaves were also extracted separately with ether, acetone and 5 per cent hydrochloric acid by shaking in cold. None of the fractions gave any precipitate with the alkaloidal reagents. Petroleum ether and ethereal extracts contained mainly the green colouring matter along with little jelly-like substance, while all the remaining extracts contained a light coloured substance which foamed heavily in water.

From the absolute alcoholic extract and that of rectified spirit extract, on concentration and cooling, needle-shaped, colourless crystals of a substance could be separated. This substance has been finally identified to be a non-active, non-reducing sugar, *d-mannitol* as confirmed by mixed melting point and infra-red spectra.

The other compound that gave much foam in water has been provisionally named as *glycoside-saponion fraction* on account of its action on the frog's heart and the foaming properties. Further work on the latter fraction is still in progress.

During the next 6 months, i.e. up to 31st March 1957, more work will be done to confirm the action of glycoside-saponin fraction on frogs and other animals.

10. Inquiry into the influence of antirheumatic substances on the hypophyseo adrenocortical axis under Dr. G.K. Karandikar at Medical College, Baroda.

This investigation was undertaken to inquire into (A) the mechanism of action of anti-rheumatic substances, especially with a view to correlate the probable mediation of their effects through the hypophyseo-adrenocortical axis. Van Cauwenberge *et al.* (1951) as also Hetzel and Hine (1951) reported a depletion of cholesterol and ascorbic acid contents of the adrenal glands of rats given sodium salicylate, suggesting the possibility that salicylates stimulate the anterior-pituitary to excite the adrenal cortex, resulting in the liberation of corticoids which relieve rheumatic or rheumatoid symptoms of patients. They suggested the question whether or not other anti-rheumatic substances act in a similar fashion, (B) to examine this, it was proposed to study the action of some antirheumatic substances on the pituitary and adrenal system, using sodium salicylate as a standard anti-rheumatic substance.

White rats bred in the laboratory were used. As indices of adrenocortical activity, (C) (i) the cholesterol and ascorbic-acid contents of the suprarenal glands, (ii) the total circulating eosinophil count, and (iii) the histological pattern of lymphoid tissue were observed. Standard techniques were followed: (D) for hypophysectomy, Bulbering's modification of Smith's method, Bulbering (1938) for cholesterol (King, 1951) and ascorbic acid (Roe and Kuether, 1943) estimations. (E) Cholesterol and ascorbic-acid content of the adrenal glands of normal rats and also their total eosinophil count and histological picture of lymphoid tissue have been done.

Results

Mean value of ascorbic acid: 136.1 mg./100 g. of the adrenal gland
S.D. ± 18.65

Mean value of cholesterol : 4.470 g./100 g. of the gland
S.D. ± 1.030

Mean eosinophil count : 44/Cu mm.

(F) Standard observations with sodium salicylate have been made with the largest therapeutic daily dose used in human beings on body-weight basis. (i) Dose.—0.3 g./kg. i.p., and observations made after 100 minutes.

Mean value of ascorbic acids : 855.7 mg./100 g. of the gland
S.D. ± 19.66

Mean value of cholesterol : 4.388 g./100 of the gland
S.D. ± 1.340

Mean eosinophil count 39/Cu mm.

While the ascorbic-acid level of the adrenal glands is significantly lower than normal, the cholesterol content of the adrenal glands and the

total eosinophil are not significantly affected and, therefore, all the procedures have been repeated at an interval of six hours after the drug administration.

(ii) Mean value of ascorbic acid: 92.7 mg./100 g.

S.D. $\pm$ 13.6

Mean value of cholesterol: 4.547 g./100 g.

S.D. $\pm$ 1.006

Mean eosinophil count: 69/Cu mm.

The six hours picture is not significantly different from the 100 minutes picture.

(G) Observations with butazolidine at 20 mg/kg. and 50 mg./kg. doses orally. Doses have been made at 2 hours interval.

Results :—(i) Butazolidine : 20 mg/kg. orally.

Mean value of ascorbic acid: 161.2 mg/100 g.

S.D. $\pm$ 27.32

Mean value of cholesterol: 4.744 g/100 g.

S.D. $\pm$ 2.184

Mean eosinophil count: 38/Cu mm.

None of the observations are significantly different from the normal and, therefore, observations were made with a higher dose.

(ii) Butazolidine: 50 mg./kg. orally.

Mean value of ascorbic acid: 145.7 mg./100 g.

S.D. $\pm$ 20.71

Mean value of cholesterol: 3.394 g./100 g.

S.D. $\pm$ 0.9795

Mean eosinophil count: 47/Cu mm.

The cholesterol content of the suprarenal glands is significantly lower than normal, while the ascorbic-acid content of the suprarenal glands and total eosinophil count are not affected. These observations are therefore, being repeated at 4 hour and 6 hour intervals.

Similar studies with cincophen and amidopyrine are being made. The work will then be extended to (H) (i) gamma resorcylate, (ii) colchicine, and (iii) glycerrhiza. Work with patients has not yet started in earnest. Preliminary studies of E.S.R. and 17-ketosteroids have, however, begun. The E.C.G. machine has arrived. The Ayurvedic preparations are under preparations at the SUPAR Unit Rasa Shala. The work is being continued.

11. Enquiry on anti-convulsant and antifibrillatory action of drugs in relation to acetylcholine synthesis under Dr. B.C. Bose at the M.G.M. Medical College, Indore.

Effect of some of the common anti-convulsant drugs, such as dilantin sodium, thiantoin sodium, mesantoin and tridione, on the synthesis of acetylcholine in brain and heart tissues was investigated with a view to study their mechanism in arresting the irregularities of rhythm and electrical discharge. In one set of chronic experiment four groups of rats were kept on a synthetic diet containing 0.02 per cent anti-convulsant drugs, for about 6 weeks, before being sacrificed for estimating acetylcholine content in brain and heart tissues. The fifth group without any drug served as control. The tissue extract were assayed biologically on frog rectus, against the standard acetylcholine-chloride solutions.

The effect of these anti-convulsant drugs was also investigated in a series of acute experiments in which acetylcholine content of one half of cerebral cortex, removed before the intraperitoneal injection of the drugs, was compared with that present in the other half removed after $1\frac{1}{2}$ to 2 hours of the injection of the drug. Effect of these drugs on the synthesis of acetylcholine in heart, removed after the final ablation of brain in these rats, was also determined and compared with the normal values.

Both these chronic and acute experiments showed that the anti-convulsant drugs, in general, decrease acetylcholine content in the brain and heart tissues. The percentage inhibition of acetylcholine synthesis in these organs was found to be proportional to their anti-convulsant effects. Masantoin, milontin and thiantoin sodium, being more potent anti-convulsants, caused a more marked decreased in acetylcholine synthesis in both these organs. The arrest of hyperexcitability and abnormal electrical discharge seems to be regulated by the rate of synthesis of acetylcholine, which in turn, is influenced by these anti-convulsant drugs.

12. Inquiry on canine and human gastric function in summer and winter under Dr. B.S. Kahali at the S.M.S. Medical College, Jaipur.

HUMAN GASTRIC FUNCTION

Ten healthy volunteers without any digestive complaint of age group 18 to 28, were taken for investigation. The secretory function of the stomach was evaluated by the fractional test-meal method. Three test meals, oat gruel meal (600 c.c.), alcohol meal (100 c.c. of 7 per cent alcohol) and pectin meal (750 c.c. of 2 per cent pectin, sweetened with suger 3.5 per cent with pH adjusted to 6.5 with 0.1 N. NaOH) are used

to find out the most suitable test meal for the purpose. Alcohol meal was found to be the most suitable.

Results during Rains (mid June to mid August 1956) :—

All Figures are in terms of N/10 HCl ml per cent of gastric contents.

(1) *Fasting contents*.—Quantity ranged from 12 to 110 ml., average 81.5 ml. Free acidity ranged from 0 to 31 ml., average 16.3 ml. Total acidity from 8 to 68 ml. average—29.5 ml. Total chloride from 40 to 150 ml., average 63 ml.

(2) *Of Alcohol test meal*.—Average free acidity ranged from 21 to 54 ml. Peak of free acidity was attained 60 minutes after the meal.

Average total acidity ranged from 31 to 70 ml. and total chloride from 55 to 100 ml.

The suitability of using fresh egg white as a substrate for pepsin to estimate peptic activity on the principle of Anson Mirsky's method of estimating the "tyrosine value" of peptic digest is being investigated.

BLOOD CHEMISTRY

Serum chloride has been estimated and ranged from 90 to 115 milliequivalents liter. CO_2 content of the arterialized blood was estimated by the Vanslyke method and ranged from 42.2 to 52.1 ml. p.c.

Motor function has been investigated by fluoroscopy and radiography after barium meal under X-Ray. All the stomachs showed slight hyper-motility and emptied by 2 hours.

CANINE GASTRIC FUNCTION

In one dog a gastric pouch by the Pavlov's technique has been done. The Secretory activity of the pouch is not yet quantitatively satisfactory.

13. Study of physiology of the nervous system under Dr. B.K. Anand, at the All-India Institute of Medical Sciences, New Delhi.

This study was conducted last year under the enquiry entitled "higher nervous control over visceral activity". From April this year a research unit has been formed for a coordinated study of physiology of the nervous system.

I. SURGICAL AND ELECTROLYTIC LESIONS OF THE FORE-BRAIN STRUCTURES.

Twenty eight monkeys (macacus) and twelve cats were used, out of which twenty six monkeys and ten cats survived the operation.

Bilateral surgical ablations involving extensive areas of either the frontal or temporal lobes were produced by aspiration; ten monkeys and three cats had lesions of the frontal lobes, and three monkeys and two cats had lesions of the temporal lobes. Thirteen monkeys and five cats had restricted bilateral electrolytic lesions produced stereotaxically; 4 monkeys and 2 cats had these lesions to the amygdaloid nuclei; 4 monkeys and 1 cat to the hippocampus and periamygdaloid regions, 3 monkeys and 1 cat to the posterior orbital cortex, and 2 monkeys and 1 cat to the anterior cingulate gyrus. These animals were studied both pre- and post-operatively as regards their behaviour, food intake, cardiovascular and respiratory responses, rectal temperature, blood sugar and sodium estimations, and eosinopenic response to stress. The brain lesions were finally studied both macro- and microscopically.

The following conclusions were drawn :—

(1) *Affective behaviour*

Temporal lobe lesions.—Electrolytic lesions in the amygdaloids nuclei and periamygdaloid and hippocampal regions made both monkey and cats fearless, non-aggressive and calm. We have previously reported fearfulness from stimulations of amygdaloid regions and their lesions may, therefore, be expected to result in loss of fear. These animals also lost power of distinction between edible and non-edible objects. Extensive surgical temporal lobectomies, in addition, produced a loss of sense of localization in space and localization of direction of sound.

Frontal lobe lesions.—After extensive surgical ablations of the frontal lobes, including the posterior orbital cortex and the anterior cingulate gyri the animals appeared confused and somewhat apraxic. They were docile and rather shy of test objects. Some of them also lost distinction between edible and non-edible objects. Animals with restricted electrolytic lesions to the anterior cingulate gyri mainly showed fearlessness, docility and hypomotility. Stimulation of anterior cingulate previously has been shown to produce viciousness. Electrolytic lesion to the posterior orbital cortex, on the other hand, made the animals restless but less fearful in contrast to stimulation of orbital cortex reported previously as quietening the animals. Some monkeys developed hyperactivity after frontal lobe lesions, and majority of them had lesions involving both the posterior orbital cortex as well as head of caudate nucleus. This hyperactivity is possibly linked more with damage to the head of the caudate than to the posterior orbital cortex. The detailed results have been sent for publication as under :—

‘Changes in the affective behaviour produced by lesions in the frontal and temporal lobes’—*Indian Journal of Medical Research*.

(2) *Food intake*—Of these animals showed marked and varied changes. Frontal lobe lesions including the posterior orbital cortex, or restricted to orbital cortex, led to a decrease in food intake; while those which spared the posterior orbital cortex led to an increase in food intake. Lesions restricted to anterior cingulate did

not change the food intake. Lesions in the amygdala and periamygdaloid regions produced aphagia or marked decrease in food intake lasting for a few days, after which the intake returned to normal levels. Extensive temporal lobe lesions produced an increase in food intake, preceded by an initial decrease if amygdala also was involved. Changes in food intake tended to return to normal after some time.

Such changes were more marked in monkeys than in cats, and were never so pronounced as after hypothalamic lesions. Pre-operative daily variations in food intake tended to disappear after these lesions. It had been previously reported that there are two opposing mechanisms for regulation of food intake in the hypothalamus and that at least in primates these are influenced from 'higher' levels. The results of present study corroborate that. It is concluded that fore-brain structures modify food intake through a discriminating mechanism ('appetite'), while the hypothalamus contains the primitive urge ('hunger'). The fore-brain mechanism can modify the hypothalamic mechanism. The detailed results have been sent for publication as under:—

'Higher nervous control over food intake'—*American Journal of Physiology*.

(3) *Circulatory changes.*—

(i) *Heart rate.*—Extensive surgical lesions to the frontal lobes showed an increase in heart rate in most of the animals. Restricted electrolytic lesions in these structures, on the other hand, showed only negligible changes in the heart rate. Temporal lobe lesions (both surgical and electrolytic) involving the amygdaloid and periamygdaloid regions produced a decrease in heart rate.

(ii) *Blood pressure.*—Changes, on the other hand, were not very marked. Frontal lobe lesions mainly showed a slight drop in blood pressure, while temporal lobe lesions showed a definite rise in the B.P. Previous stimulation studies had brought out a rise in B.P. from frontal lobes and a fall from temporal lobes, and opposite type of effects could thus be expected from lesions of these regions. The changes in heart rate could be explained as compensatory to changes in B.P.

(4) *Rectal temperature.*—Majority of animals with frontal lobe lesions had some drop in rectal temperature, which may be due to vasodilatation (fall in B.P.) and loss of heat from skin. Temporal lobe lesions did not show any consistent change.

(5) *Respiratory changes.*—Respiration rate was generally decreased both after frontal and temporal lobe lesions. Stimulations had previously shown an increase from frontal lobe and a fall from temporal lobes.

(6) *Gastro-intestinal tract.*—A post-mortem examination of the animals with these lesions showed pathological conditions of the mucosa. Some merely showed hyperæmia, but others had small hæmorrhages or even ulcers. Two had some sloughing of the mucous membrane.

One animal after electrolytic lesions in the hippocampus developed marked ballooning of the G.I. tract which was confirmed by x-ray examination with Ba. meal. This ballooning lasted for 2 to 3 months and then gradually disappeared.

(7) *Blood sugar* level changes after both frontal and temporal lesions were quite variable. Although majority of the animals with such lesions showed some increase in the blood sugar level, some animals with similar lesions developed a decrease.

(8) *Blood sodium* content also showed similar variations as blood sugar; majority showing a slight rise in it. This is in contrast to the work reported by Heath.

(9) *Blood eosinophile counts*.—The eosinopenic responses to stress stimuli were of the same order both before and after frontal and temporal lesions, indicating that these regions probably do not take part in the secretion of ACTH through mediation of stress. The detailed results of these studies have been sent for publication as under :—

‘Changes in visceral and metabolic activities after frontal and temporal lobe lesions’—*Indian Journal of Medical Research*.

II. NERVOUS CONTROL OVER GASTRIC SECRETION STUDIED BY POUCH METHOD.

The gastric secretion has been studied by collecting it from pouches separated from the rest of the stomach in cats. Stimulation of different hypothalamic regions were carried out in unanæsthetized animals by implanted electrodes. It was observed that the stimulation of the pre-optic region and the antero-medial hypothalamus led to a marked increase in the acid and pepsin secretion and also produced acute hæmorrhagic ulcers. Very often hæmorrhages were so marked as to lead to the death of the animal. The detailed results have been sent for publication as under :—

‘Effect of electrical stimulation of hypothalamus on gastric secretory activity and ulceration’—*American Journal of Physiology*.

Further studies are now being conducted with the stimulation of different limbic lobe structures.

III. EFFECT OF RESERPINE (SERPASIL) ON BLOOD PRESSURE RESPONSES EVOKED FROM HYPOTHALAMUS.

This study, which was being conducted in association with Dr. C.L. Malhotra, Professor of Pharmacology, has been concluded. It was observed that reserpine increases the threshold of stimulation for producing a hypertensive response, and decreased the threshold of stimulation for producing a hypotensive response. Thus, it not only

depresses the sympathetic centres, but also facilitates the parasympathetic centres. The results have been sent for publication as under —

‘Effect of Reserpine (Serpasil) on blood pressure responses evoked from hypothalamus’—*British Journal of Pharmacology and Chomotherapy*.

IV. ACETYL-CHOLINE AND GLUTATHIONE ESTIMATIONS IN HYPOTHERMIA.

This study has also been conducted in association with the Pharmacology Department. In 20 dogs, hypothermia was produced by venous shunt through ice and surface cooling. After the temperature came down by 10°F. to 12°F. the heart and different parts of brain, especially hypothalamus, were taken out and their acetylcholine and glutathione contents estimated. Some dogs with hypothermia were also given serpasil or largactil. These studies were carried out to find out any explanation for ventricular fibrillation or irreversible hypothermia. The results indicate a decrease in acetylcholine and glutathione in heart and hypothalamus after hypothermia. The results are being prepared for publication.

V. HYPOTHALAMUS AND REGULATION OF WATER INTAKE.

This is being studied in female albino rats of Kasauli strain. So far 40 rats have been operated upon. Their food and water intake is recorded for few weeks before operation. Then small bilateral, well localized electrolytic lesions are produced stereotaxically in different hypothalamic regions, and food and water intake recorded post-operatively. Marked variations in water intake have been observed. This study is still incomplete and is being carried on.

VI. PHYSIOLOGICAL CONNECTIONS BETWEEN THE CEREBELLUM AND THE LIMBIC LOBE STRUCTURES.

Evoked potential studies are being carried out to map connections between the cerebellum and the limbic lobe structures in dogs. So far 30 animals have been studied. They are anaesthetized by intraperitoneal Dial (Ciba), and the brain is exposed. Multilead electrodes are then implanted in various limbic lobe structures, and connected to the cathode ray oscilloscope. Various regions of the cerebellum are then stimulated, and action potentials recorded photographically. The results obtained so far indicate that in contrast to the connections between the neocerebellum and the neocortex demonstrated previously by various workers, there are abundant connections between the flocculonodular lobe of the cerebellum and the various limbic lobe structures. These to our knowledge have not been so far reported by any one. This work is continuing and many more experiments will have to be done.

14. Inquiry on the role of Electrolyte imbalance in hypertension under Dr. Inderjit Singh at the S.N. Medical College, Agra.

It is well known that sodium retention causes hypertension, and further that this action of sodium is peripheral. But it is not known

how sodium produces this effect. The effect of sodium, potassium, and calcium was tested directly on the contractile mechanism of smooth muscle of the blood vessels. It has been found that sodium and calcium have constrictor effect and potassium, a dilator effect. Further, it has been found that cholesterol has a constrictor effect on the blood vessels.

These researches have been extended to determine the effect of adenosinetriphosphate on smooth muscle, in general, and on that of blood vessels, in particular. Experiments were performed to test the effect of adenosinetriphosphate on active relaxation of smooth muscle and on blood vessels.

To test the effect of adenosinetriphosphate on active relaxation, in the first series, 12 experiments were performed on transverse pieces of frog's stomach. In 3 experiments, 1.5 p.c. ATP with 0.005 M Mg; was used. There was no significant relaxation. In 3 experiments, the ATP concentration was reduced to 0.5 p.c. with 0.005 M Mg there was no relaxation. In the next 3 experiments, 1.5 p.c. ATP with 0.05 M Mg was used : the muscles relaxed by 6, 12 and 9 p.c. In the last 3 experiments 0.5 p.c. ATP with 0.05 M Mg was used : the muscles relaxed by 20, 20 and 11 p.c., respectively. Thus, the optimum concentration of ATP was found to be 0.5 p.c. and Mg 0.05 M. Calcium opposes the effect of magnesium in causing relaxation.

In the next series, 66 experiments were performed, with varying concentrations of magnesium, calcium and ATP, but no relaxation was observed. We are at present unable to account for this. Experiments are further proceeding.

To test the effect of ATP on the contractile mechanism of the smooth muscle of blood vessels, the hind limbs of small dogs were glycerinesed, and perfused, with ATP. Only two experiments have been performed, and the results are inconclusive. Further experiments are being done.

15. Inquiry on the treatment of cirrhosis of liver with *Eclypta alba* leaf extract under Dr. R. Subramanian at the Madras Medical College and Government General Hospital, Madras.

Eclypta alba leaf extract has been used in the treatment of cirrhosis of liver in adults for the last 2-1/2 years. The total number of cases treated with *Eclypta alba* alone is 32. It has been found that early cases respond by the clearance of the ascites, improvement in the appetite and by increase in weight. In some cases even after number of tappings, as many as 11, the ascites was not cleared ; in one case, while the patient was tapped 11 times without the administration of *Eclypta alba* leaf extract, the ascites was cleared and the patient is now clinically symptom-free. For the last 6 months, we have been using in the wards of one of our colleagues for control purposes. Seven cases were admitted into the wards. Of these one died and the remaining 6 are all being tapped in shorter and shorter intervals. Urine out put is

same as on admission and the œdema of the legs and ascites are persisting. The diet has been common for the cases under my care as well as under my colleague's care. The only significant difference is my patient is getting *Eclypta alba* leaf extract in addition.

Total number of patients treated as in-patients ... 7

Investigations done :

1. Total : W.b.c.
R.b.c.
Hæmoglobin per cent.
2. Estimation of serum protein : Albumin
Globulin
3. Ascitic fluid—protein content
malignant cells.
4. Liver-function tests : Thymol turbidity
Icteric index
vanden berg
5. Liver biopsy
6. Blood V.D.R.L.
7. X-ray chest.

Total number of patients proved to be cirrhosis liver by
liver biopsy ... 6

Total number of patients proved to be fatty liver by liver
biopsy ... Nil

Treatment adopted for patients having cirrhosis liver.

1. Tapping
2. Mist. Wodelia Calendula oz. iii, oz i t.d.s.
3. Protein food—1 measureful orally, 3 times a day
4. Crude liver with folic acid i.v. daily with glucose.
5. Achromycin 500 mg. i.v. daily for 3 days with glucose and drip in cases of cholæmia.

The following diet is given to them :—

- | | |
|--------------------------|--------------------|
| 1. Bread 8 oz. | 4. Oranges 10 |
| 2. Skimmed milk 3 points | 5. Vegetable 6 oz. |
| 3. Meat or liver 4 oz. | 6. Sugar 1 oz. |

Total number of patients treated as out-patients ... 6

Treatment adopted :

1. Mist Wodelio Calendula oz. iii oz. 1 t.d.s.
2. Crude liver with Vit. B₁₂ and folic acid 2 cc. daily i.v. with glucose.

SUMMARY

Total number of cases treated	...	7
Total number of cases discharged	...	4
Number of cases as in-patients 3 being treated.		

All the treated cases have come from poor families except one from a fairly rich family who gave history of chronic alcoholism. All of them were on low-protein diet. The onset of signs and symptoms were gradual, none one took initial care to get themselves treated in the beginning.

All the cases responded well to the administration of *Mistura extractum Wodelia calendula* orally; œdema of the lower extremities cleared, the interval of subsequent tapplings became prolonged, i.e. from 7 days to 14 days. Appetite improved, urine output increased.

Three cases which have gone into cholæmia recovered by achromycin therapy i.v.

One case previously tapped 5 times, was re-admitted for recurrence of ascites, was subsequently tapped 6 times but after the 11th tapping, the patient remained free from ascites for one-and-a-half months, appetite improved, put on weight and discharged.

Total number of patients kept as control cases in Dr. K.S. Sanjivi's Unit	...	7
---	-----	---

Investigations done :

Serum albumin
Globulin
Icteric Index
vanden berg
Thymol turbidity
Alkaline phosphatase

Treatment adopted :

- | | |
|---|--------------|
| 1. Tapping | } i.e. daily |
| 2. Liver extract 2 c.c:
<i>plus</i> | |
| 3. B. complex 1 c.c. | |
| 4. Mist. acid tonic oz. iii, oz. i t. d. s. | |

SUMMARY

Out of all these patients one died. The remaining cases developed ascites within ten days of tapping. Oedema of the extremities did not clear up, urine output remained same.

16. Studies on the growth of resistance in micro-organisms against antibiotics under Dr. S.K. Bose at the Department of Applied Chemistry, Calcutta University, Calcutta.

The growth of resistance has been accounted for by two major classes of hypothesis, one class involving mutation and selection and the other involving adaptation. It may be that one or other of the hypotheses may be finally accepted or that these opposite views so expressed may simply be due to the difference in the nature of strains used by different investigators in different laboratories or in other words different mechanisms may operate in different cells to develop resistance. Our interest is, however, to investigate whether or not both the suggested mechanisms operate simultaneously in one and the same organism. Work was, therefore, carried on the following lines to elucidate the point :—

- (I) Normal distribution of resistance against penicillin.
- (II) Normal distribution of resistance against formaldehyde.
- (III) Induction of penicillin resistance by chemicals other than penicillin.

(I) *Normal distribution of resistance against penicillin.*—Studies on the normal distribution of resistance in a population indicate that a given experimental population may be considered as made up of individuals of varying degrees of resistance. All the individuals possess a given minimum of resistance while the great majority of cells an average and the minority an exceptionally high resistance. It is very curious to note the high degree of resistance possessed by this microscopic minority of the population whose magnitude of resistance depend not merely on the level of antibiotic treatment, but also on the initial total cell count. It may be taken as an indication that the mechanism which accounts for average resistance may not do so for the development of exceptionally high resistance. The probable distribution of resistance in populations of resistant cells isolated at different levels of penicillin may indicate the mechanism or mechanisms involved in the process. It is also of interest to point out that it has been argued by many workers that one or two exceptionally highly resistant cells may be present in a population whose even but rare distribution may account for emergence of resistant cells in large populations. Further work is necessary to elucidate this point because the resistant type may or may not be initially present but subsequently produced in course of cellular growth. The point can, however, be clarified by a comparative study on the frequency of occurrence of a given resistant type in the original population and that in populations subsequently raised by serial sub-cultivations.

(II) *Distribution of resistance against formaldehyde.* (a) *Mutagenic chemical.*—Studies on the distribution of penicillin resistance leads to certain observations relating to distribution of resistance. It is of interest to enquire whether or not this type of distribution represents a general one. Formaldehyde, a mutagen, is selected for the purpose.

It appears that the pattern of distribution of resistance is identical in both cases. This may be taken as an additional evidence in support of the views expressed in the earlier paragraph.

(III) *Induction of penicillin resistance by substances other than penicillin.*—In the development of resistance against penicillin, the antibiotic is supposed to act according to mutation theory, merely as a selecting agent. It is, therefore, felt desirable to enquire whether or not substances other than penicillin can induce penicillin resistance. Formaldehyde a mutagenic chemical, was selected for the purpose. It appears that formaldehyde can induce penicillin resistance in the majority of cells of a population possessing average formaldehyde resistance but not in the few cells which represent a highly formaldehyde resistant group in the population. This phenomenon is, therefore, different from that of cross-resistance. This has been found to be the case with all other isolates at different levels of formaldehyde resistance. This shows that the mechanism which accounts for the development of resistance in the majority of cells may not do so for the minority where probably a different mechanism may be involved for the development of highly resistant cells. Additional information on the induction of penicillin resistance by other mutagens may also prove helpful in this respect. The phenomenon of cross-resistance in respect of penicillin resistant cells may also throw some light on the probable mechanism or mechanisms of penicillin resistance.

VIRUS DISEASES

1. Rabies research under the Director, Central Research Institute, Kasauli.

I. *Preparation of local standard antirabic sera.*—In view of the limited quantity of W.H.O. antirabic serum standard available, a local standard was prepared in lyophilized state for future testing.

II. *To ascertain the optimal interval between serum treatment and commencement of vaccine therapy.*—Nine groups of ten guinea-pigs each were given a dose of street virus followed by serum. The different batches received vaccine treatment commencing from two hours to six days after serum treatment. No differential result was obtained probably because of the severity of the infecting dose.

III. *The influence of the route of infection with fixed virus on the virus content in sheep brain.*—Four sheep were infected subdurally with 0.4 c.c. of 1/5 emulsion of Pasteur-strain of fixed virus rabbit brain, while a similar number received 0.8 c.c. of 1:300 dilution of the same emulsion into the cisterna magna. When the sheep were completely paralysed the brains were harvested and titrated separately. The virus content of the brains from each group varied from 10^6 to 10^7 . •

IV. *Comparison of serum antibody level of monkeys receiving the same quantity of brain by a short and a long course by immunization.*—Two monkeys were injected with 0.5 c.c. of a 5 per cent. Semple vaccine

for 14 days, while another two received 1 c.c. of the same vaccine for seven days—pre-immunization blood, blood after 16 days from the commencement of treatment and 10 days after the end of treatment are being tested for the level of serum antibodies. The experiment is still in progress.

V. The nuclei of the spermatid cells of the fixed virus infected mice show round vacuoles, near the nuclear membrane. Starved mice also show the same type of vacuoles though to a much less extent.

VI. Attempts are being made to adapt freshly isolated strains of street virus to grow in trypsinized monkey kidney-tissue cultures.

VII. *Interference phenomenon between type 2 polio virus and rabies street virus.*—Sucking mice inoculated in the leg with 100,000 TD_{50} type 2 tissue culture adapted polio virus were given street virus emulsion in the same leg after 72 hours—though none of the experimental animals died, three out of the nine mice in the control group died of street virus infection. This work is being repeated.

VIII. *Interference phenomena in tissue culture.*—Monkey-kidney tissue—culture tubes to which rabies street virus had been added eight days previously were challenged with 100 TID_{50} of polio type 2 virus. There was no evidence of interference *in vitro*.

2. Polio Research Unit under Dr. P.V. Gharpure at the Grant Medical College, Bombay.

A. EPIDEMIOLOGICAL STUDIES

One hundred and sixty four cases were studied from 1-10-1955 to 31-8-1956. These were from 4 different hospitals of Bombay, and 3 cases were referred to by private practitioners. Of the 164 cases, 96 were males and 68 females. Seven cases were declared as non-polio after due investigations. Of the 157 cases, 156 were paralytic and 1 non-paralytic. Of the 156 paralytic cases 134 were spinal, 16 bulbar and 6 bulbo-spinal.

There were 15 fatal cases (9.1 per cent) of which 9 were bulbar, 5 spinal, and 1 non-polio. Among the fatal cases, only 1 was recorded in an adult and the remaining 14 were below the age of 10 years. The study of the distribution of the cases in different wards has shown that they occur in all wards though not uniformly. More cases occurred in the inland part and cases concentrated on the border line of a group of wards.

Further study of the sequence of cases in each ward has failed to show either centrifugal or centripetal spread. The epidemic appears, therefore, to be multicentric.

Of the suburbs, Kurla recorded 7 cases, Chembur 5 and Ghatkopar 4. The rest of the suburbs recorded one or two cases each.

There were 32 cases of paralytic poliomyelitis imported from a number of places outside Bombay. These places were within the radius of about 200 miles.

Studying the month-wise distribution, it is again found that the epidemic period is more or less distinct, namely, May to August.

The age-wise distribution shows that the bulk of the cases have occurred between 6 months and 4 years.

The study of children below the age of 10 years in a selected locality in Bombay was undertaken to determine the age of onset of the disease and, if possible, the mode of spread of infection. Attention was, therefore, directed to determine the number of healthy children and those suffering from minor illnesses among the families studied in the selected locality. One of the findings of this study is that the ratio of paralytic cases to all other minor illnesses is 1 to 100.

B. FIELD WORK

654 home visits were made and data and materials for study collected.

C. VIRUS STUDY

All the work was carried out in tissue culture. A spot map of Bombay City showing the prevalence of the types of poliomyelitis virus in different wards has been prepared for the first time. The distribution of the types of virus is not uniform in all the city wards, e.g., only type II is prevalent in Ward A and only type I in ward G as compared to two or more types in other wards.

Since the introduction of tissue culture the percentage of virus isolations has increased considerably. Fifty-nine isolations from 173 samples of stools collected from clinical cases in Bombay City and suburbs and 16 from 55 samples collected from imported cases (including the material received through post) have been made. Five isolations from 42 samples of stools collected from contacts of clinical cases have been done.

The study of the child population under 10 years in the selected locality enabled the Unit to survey 849 children of whom 441 were apparently healthy and the remaining 408 were having some sort of minor illness. 574 samples of stools were collected from the child population and 42 virus isolations were made. Twenty four of the isolations proved to be non-polio on serological grounds. This is an interesting finding and is likely to form the basis of future work.

This is the first time that the Unit is able to report on a group of 5 human autopsies on poliomyelitis. From the material of these 5 cases, 3 strains of virus have been isolated and all the 3 belonged to type I.

Hyperimmune type-specific sera against all the 3 types of virus have been prepared in monkeys and rabbits.

The preliminary effort to set up serological work for the detection of virus-neutralizing antibodies has been made. Seventy-three sera have so far been tested. Fifty-six of the 73 sera were collected by the finger prick method. This preliminary study has brought out one fact that over 80 per cent of the sera above the age of 9 years showed the presence of antibodies against all the three types of poliomyelitis virus.

3. Inquiry on studies on the incidence, nature and types of Cocksackie virus infections in Bombay City under Dr. N.M. Purandare at the Seth G. S. Medical College, Bombay.

(1) *Collection of material.*—Twenty-one faecal samples were collected from patients, clinically diagnosed as suffering from poliomyelitis and admitted to the Bai Jerbai Wadia Hospital for Children, Bombay-12. A collection of 16 autopsied brain and/or spinal cord samples from suspected cases of encephalitis, was made with a view to isolating, if possible, any other mouse adaptable virus. About one thousand fruit flies were collected from 32 different localities at different times, thus making a total of 317 cases from the beginning of the work on the virus investigation.

(2) Following samples were investigated for the presence of Cocksackie viruses by subcutaneous inoculations into new-born albino mice.

- (i) Twenty-one faecal samples from poliomyelitis cases, and
- (ii) about six hundred fruit flies suspensions in 14 lots.

Out of these, positive isolation from one faecal sample was made. Histopathologically the strain appears to belong to Dalldorf's group A. The strain is being typed antigenically by neutralization tests-with the available typed antisera.

(3) Attempts to isolate any mouse adaptable viruses from 16 brain and/or spinal cord samples and about six hundred fruit flies samples gave negative results by intracerebral inoculations into adult albino mice.

(4) Cultivation of Cocksackie virus strains in chick embryos : Four of the untypable strains (235, 233, 165 and 126) of Cocksackie viruses isolated previously were inoculated into chick embryos by both chorio-allantoic as well as by amniotic route following the methods described by Slater and Syverton (*Proc. Soc. Exper. Biol. & Med.*, **74** 509-510, 1950).

Positive results up to the second alternate mouse passage were obtained from three of the strains (235, 165 and 126) while the fourth one could not be propagated further after the first passage.

(5) Tissue culture studies :

(a) *Monkey kidney cell culture in stationary tubes.*—Attempts to recover poliomyelitis virus from 21 faecal samples collected for the investigation of Cocksackie viruses, yielded negative results by inoculations into monkey kidney tissue culture tubes.

(b) *Mouse and chick tissues*.—Successive attempts were pursued to grow the embryonic mouse and chick heart tissue, embryonic mouse and chick pulp and mouse placenta and diaphragm, either on plane glass surface or on plasma clots following the routine methods. Experiments were also carried to trypsinize embryonic mouse and chick tissues after beheading them. As a nutrient medium different combinations of horse serum, ox serum, calf serum, etc., with Hank's solution were tried. Results on the inoculations of Cocksackie virus strains on these growing tissues are awaited.

4. Rabies inquiry under Dr. N. Veeraraghavan at the Pasteur Institute, Coonoor.

INTRODUCTION

During recent years important contributions have been made to the subject of antirabies treatment. These include (i) the use of hyperimmune antirabies serum with and without vaccine therapy, and (ii) the use of chicken embryo-adapted rabies vaccine. It was considered desirable to investigate all these advances under a single set up with a view to assess (i) the various claims, (ii) their relative merits, and (iii) whether they hold good when Indian strains of street virus are used for challenge instead of the strains used by other workers. It was also considered essential to re-assess the value of 5 per cent space properly Semple vaccine in experimental rabies infection employing the latest techniques. The important result of treatment with (i) antirabies serum alone, (ii) 5 per cent Semple vaccine, (iii) a combination of serum and vaccine, and (iv) high egg passage (HEP) chicken embryo-adapted rabies vaccine with and without serum, are briefly summarized below.

MATERIALS AND METHODS

Challenge.—In order that our results may be comparable with those reported by others the lyophilized canine submaxillary gland suspension of the NYC strain of virus was mainly used in this study. The Marimuthu and Masuli strains obtained locally were also used in some experiments. The number of intramuscular LD₅₀ used as challenge in each experiment was determined.

Antirabies serum.—Lederle's antirabies serum (L), 1,000 units per 10 c.c. as well as serum prepared at the Institute were tried. The dosage employed was 0.25 c.c. per pound of body-weight. In each experiment where serum was used the number of neutralizing doses (ND) administered in respect of the challenge was determined.

Vaccine.—5 per cent Semple vaccine prepared at the Institute was used.

HEP vaccine.—Lederle's high egg passage chicken embryo-adapted rabies vaccine was used.

Results

1. ANTIRABIES SERUM THERAPY

(a) *Pre-infection treatment with serum.*—Antirabies serum (L), 0.25 c.c., (ND=5,270), had no protective value when given i.m., 4 days before a challenge of 8 LD₅₀ of NYC.

A heavy dose of serum (L), 1 c.c. (ND=21,080), had no saving effect when given i.m., 5 minutes before a challenge of 8 LD₅₀ of NYC.

Unconcentrated antirabies horse serum (PIC), 2.5 c.c., (ND=9,900), conferred no protection when given into the neck muscles just before challenge.

Serum (L), 0.2 c.c., (ND=2,416), had no saving effect when administered intracerebrally just before a challenge of 11.3 LD₅₀ of NYC.

(b) *Post-infection treatment with serum.*—Serum (L), 0.25 c.c. (ND=1,040), infiltrated locally into the site, 1 hour after a challenge of 8 LD₅₀ of NYC, conferred no protection.

Serum (L), 0.25 c.c. had no saving effect when given i.m., 1 hour after a challenge of 16, 8 or 1.3 LD₅₀ of NYC.

The pseudoglobulin and euglobulin concentrates of hyperimmune antirabies serum from horses, when given in 0.25 c.c. doses, i/m., 1 hour after a challenge of 3.2 LD₅₀ of NYC, had no protective value.

Antirabies serum (L) raised in rabbits, 0.25 c.c. (ND=1,862), given i.m., 1 hour after a challenge of 9 LD₅₀ of NYC, had no saving effect.

There was some evidence of protection against a challenge of 11.3 LD₅₀ NYC, when serum alone from guinea-pigs, which were immunized with vaccine and had survived a challenge with NYC, was given in 1 c.c. doses (ND=182), i.m., 1 hour after challenge.

There was no protection when serum (L), 0.25 c.c., (ND=3,165), was given i.m., 1 hour after a challenge of 12.6 LD₅₀ of Marimuthu strain.

In all, the animals treated with serum, the incubation periods were considerably prolonged and majority of them died of furious rabies.

2. 5 PER CENT PHENOLIZED ANTIRABIES VACCINE THERAPY

(a) *Pre-infection treatment with vaccine.*—There was no mortality among animals given 7 × 1 c.c. of vaccine, daily, s.c. when the treatment was started 7 days before a severe challenge of 16 LD₅₀ of NYC.

When the treatment was started 7, 5, 3 and 1 day before a challenge of 8 LD₅₀ NYC, the mortality rates were 0, 13, 33 and 88 per cent, respectively. Thus, about two-thirds of the animals could be saved when the treatment was started 3 days before challenge.

There was excellent protection among animals treated with 7 × 0.3 c.c. of vaccine, daily, s.c. when the treatment was started 7 days

before a challenge of 8, 11.3 and 12.7 LD₅₀ of NYC. The total of 2.1 c.c. given corresponds to a class III dosage of 140 c.c. for a human being.

The protection was about 50 per cent when the 2.1 c.c. was given in a single dose, 7 days before challenge. The results were better when the vaccine was given in 2 doses, 7 and 1 day before challenge. These findings indicate that the same quantity of vaccine administered in multiple doses confers better immunity than when given in 1 or 2 doses.

There was no protection when treatment with 7×0.3 c.c. vaccine, daily, s.c. was started 4 days and 4 hours before challenge.

When the total quantity of vaccine administered was halved by giving 7×0.15 c.c. daily, s.c. there was very little protection even against a mild challenge of 1.3 LD₅₀.

There was a mortality of 33 per cent. among animals given 7×0.3 c.c. vaccine, daily, s.c. when the treatment was started 7 days before a challenge of 12.6 LD₅₀ of Marimuthu strain of virus.

(b) *Post-infection treatment with vaccine.*—There was some evidence of protection among animals treated with 7×1 c.c. of vaccine, daily, s.c. even though the treatment was started 1 hour after challenge. The mortality rates were 60, 73 and 68 per cent against 8, 11.3 and 16 LD₅₀, respectively.

There was no protection among animals treated with 7×0.3 c.c. of vaccine, daily, s.c. when the treatment was started 1 hour after challenge. There was a mortality of only 70 per cent against the same challenge when the 2.1 c.c. of vaccine was administered in 14 equal doses of 0.15 c.c.

There was no protection among animals treated with 7×1 c.c. or 7×0.3 c.c. of vaccine, daily, s.c. when the treatment was started 1 hour after a challenge of 12.6 LD₅₀ of Marimuthu strain of virus.

3. COMBINED SERUM AND 5 PER CENT PHENOLIZED VACCINE THERAPY

The mortality was the same when 0.25 c.c. neat or 1:10 dilution of serum (L) was given, i.m., along with vaccine, both being started 1 hour after challenge.

The mortality was 53 per cent among animals given 1 c.c. of serum (ND=12,080), i.m. and the vaccine, both being started 1 hour after a challenge of 11.3 LD₅₀, compared with a mortality of 73 per cent among the group treated with the vaccine alone. This would suggest that the results are not proportionately better when larger doses of serum are given.

When the challenge is severe, the results with combined serum and vaccine therapy are not markedly superior to those obtained with vaccine treatment alone.

In animals treated with 7×0.3 c.c. of vaccine and serum (L) in varying doses, both being started 1 hour after infection, the best results

were obtained in the group which received 0.25 c.c. of a 1:5 dilution of the serum along with the vaccine. The results were poor with larger as well as smaller doses of serum. These findings suggest that an optimum relation between the dosage of serum and vaccine has to be maintained for good results.

An evaluation of the administration of serum, 0.25 c.c. of a 1:5 dilution and vaccine, 7×0.3 c.c., at varying intervals indicates that the best results are obtained when serum and vaccine are started 1 hour after exposure. Good results are obtained when serum is given 1 hour after challenge and vaccine started 24 hours after challenge. When vaccine therapy is postponed the results are not satisfactory. There does not appear to be any advantage in giving serum 24 hours and vaccine 48 hours after challenge. The results, however, suggest that combined serum and vaccine therapy may be of some value when started up to 6 days after challenge.

The results of treatment with vaccine and serum (PIC) against NYC, Marimuthu and Masuli strains confirm the observations with Lederle serum and NYC challenge that (i) better results are not obtained with larger doses of serum, and (ii) there is an optimum level of serum for a particular dosage schedule of vaccine treatment.

4. TREATMENT WITH HEP VACCINE ALONE AND WITH SERUM

There was very good protection among animals treated with HEP vaccine, 0.25 c.c. of a 10 per cent given i/m, 21 and 12 days before a challenge of 11.3 LD₅₀ NYC and 12.6 of Marimuthu strains.

There was a mortality of 33 per cent among animals given 0.25 c.c. of serum (L), s/c, 24 hours after infection and HEP vaccine 24 hours later, when the challenge was 11.3 LD₅₀ of NYC. With the same combined treatment the mortality was 100 per cent with an almost indential challenge of 12.6 LD₅₀ of the Marimuthu strain of virus.

5. Study of lymphogranuloma psittacosis group of viruses with special reference to ornithosis under Dr. J.B. Shrivastav at the Medical College, Nagpur.

Last year, a fair number of pigeons showed a titre up to 1:40, while none of the parrots investigated showed the presence of antibodies. The survey was continued this year. With a view to isolate local strains of 'ornithosis' virus, and on the basis of our earlier experience (Miles and Shrivastav, 1951, *Jour. Animal Ecology*, **20**, No. 41) thirteen fledgelings of green parrakeet (*Psittacula krameri*) were caught within a radius of 10 miles from Nagpur. Their sera were collected immediately after catching, and at intervals of a month for two months. These thirteen sets of sera (three from each bird) were tested for the presence of antibodies of psittacosis ornithosis virus. Eight sets were negative, four sets gave a positive complement fixation in a titre of 1:10, and one set in a titre of less than 1:5. The positive sets did

not show any rise in titre in the second and third samples. The minimum diagnostic titre being 1 : 5, four birds out of thirteen were considered positive, but no virus could be isolated from the pooled organs of these birds. It is interesting to note that the parrot population appear to be less infected than the pigeon population.

This can be explained by the fact that the psittaciforms investigated were the wild green parrakeet (*Psittacula krameri*), while the columbiforms, though caught in the field, were the domesticated (*C. livia* and *C. leuconota*) pigeons reared under non-too-hygienic lofts of bird dealers and fanciers. It will be fanciers to find out the spectrum of infection in the wild pigeon population (*C. oenas* and *C. palumbus*) of the locality.

II. *Antigenic relationship of psittacosis and L.G.V. virus.*—Last year, on the basis of absorption and quantitative C. F. tests done on a high titre human L.G.V. serum, it was established that the group specific heat stable antigen is 3 to 7 times more on the psittacosis than on the L.G.V. virus. On referring this work to Prof. J.A.R. Miles, Professor of Microbiology University of Otago, New Zealand, he remarked 'with regard to your other paper, it is very interesting but at present the work is liable to criticism. For completeness a similar series of tests with a psittacosis antiserum is necessary'. Sir Macfarlane Bernet, who visited us in December, 1955, also held a similar view. To obtain a high titre human psittacosis antiserum was a difficult proposition and hence an alternative natural host of the virus, i.e. pigeon, was used for obtaining a high titre immune serum. Three adult pigeons, which *ab initio* did not show any antibodies against psittacosis virus, were given four injections of rich yolk sac material of psittacosis virus. The first dose was 1 c.c. of formalized (0.15 per cent neutral formaline) unheated material, while the subsequent doses were of 1 c.c. of live material suspended in normal saline. The injections were given in pectoral muscles at an interval of two weeks. Blood samples were collected from these birds immediately before the administration of the injections. The titres recorded are shown in the table :—

On reaching a fairly high titre at the end of 16 weeks, the birds were given a sudden, severe knock on the head, the blood vessels of the neck slashed by a sharp cut, and all the blood collected with head kept in inverted position. 7 c.c. 5 c.c. 5 c.c. of serum could be obtained from the three birds. Pooled sample of the 1st and the 3rd bird gave a C.F. titre of 1 : 2560. This pooled serum is being used for absorption and quantitative C.F. test on the same lines as done with the human L.G.V. serum last year. The Table given above shows some interesting features :—

(i) Some pigeons (bird No. 2) are refractory and will not respond to immunization in spite of the heavy dose of the virus. Perhaps those are the birds that show native, innate resistance of genetic origin, even in the complete absence of antibodies.

(ii) Though no antibodies could be detected after the administration of formalized preparation, yet the dose protects the birds from a heavy dose of live material.

Interval in weeks, with titres.

Number	Initial	1D I *	2D I *2	3D I *4	4D I *6	8	10	12	14	16	P.M. spleen	Smear virus
1/2712	...	...	...	...	1 : 5	1 : 160	1 : 640	1 : 640	1 : 2560	1 : 2560	+	-Ve
2/2779	...	...	...	...	...	...	...	...	...	...	-Ve	-Ve
3/2789	...	...	...	...	...	1 : 40	1 : 40	1 : 320	1 : 1280	: 1280	+	-Ve

(iii) The live virus perhaps is a better immunizing agent and it appears that the immune state of the birds is closely related with the physical presence of the virus in their tissues.

(iv) The immune response in pigeons is very erratic. The 'lag phase' is very long (4 to 6 weeks) in contrast to the response of other laboratory animals to bacterial preparations. It also takes a period of 14 weeks before an appreciably high level of antibodies is reached. This has been in conformity with our earlier experience with pigeons.

(v) The toxins elaborated by this group of viruses, on intramuscular injection in the pigeons, are not lethal to the birds, unlike their action on mice on intravenous injection.

(vi) The parrakeet strain of psittacosis used in this study, is not very lethal to pigeons and fails to produce active infection in them. This has been the experience of other workers also.

III. MISCELLANEOUS

(i) Strains of psittacosis virus, in form of infected yolk sacs, were supplied to the Up-graded V.D. Deptt. at the Madras Medical College and Armed Forces Medical College, Poona.

(ii) Antigens for C.F. test against L.G.V. and psittacosis infections have been supplied for diagnostic purpose to laboratories in Bombay, Poona and Madras.

(iii) A number of sera were referred to us for confirmation of diagnosis of L.G.V. and psittacosis-ornithosis infections from the V.D. clinics at Poona, and A.F.M.C. It appears that many cases with influenza-like symptoms, and positive pulmonary and radiological findings, are, in fact, infections by psittacosis-ornithosis viruses. Laboratory findings in these cases for influenza, a typical pneumonia and Q-fever, have been negative and the sera gave a positive C.F. test with group specific psittacosis antigen. The titres varied from 1 : 20 to 1 : 64 ; with evidence of rising titre in some cases. In some cases there was history of close association with poultry and pigeons.

6. Maintenance of stock cultures under Dr. (Smt.) K.J. Ranadive at the Indian Cancer Research Centre, Bombay.

The inquiry on 'Maintenance of stock culture's commenced functioning on 1st July 1956. The object of the inquiry is :

- (a) To maintain duplicate stock cultures for the Virus Research Centre, Poona.
- (b) To carry out basic and experimental studies on various cancer cell types.

Stock of three cell lines :—

- (1) Human-cancer of the cervix—strain Hela.
- (2) Human-cancer of the jaw-strain 'KB (B)',

(3) Human normal embryonic intestinal epithelium.

has been maintained in 4 oz. flat culture bottles for the last 5 months. Hela is now in its 44th passage and the other two lines are in 15th passage. The culture medium consists of 10 to 20 per cent human adult serum in Eagle's basal medium (synthetic; imported). Some cultures of Hela and KB (B) cell lines are also maintained on horse serum (20 to 40 per cent) and are in their 25th and 5th passages, respectively. 'Hela' has adapted successfully to horse serum. Recently, Eagle's basal medium has been locally prepared in the laboratory and found to give satisfactory growth response.

Detailed cytological studies of the above cell types by the use of modified glass-slip technique are in progress. It is planned to use these cell lines later for work in experimental chemotherapy.

7. Inquiry on Encephalitis in Delhi State under Dr. H.B.N. Swift at the Lady Hardinge Hospital, New Delhi.

1. This report cannot be called a comprehensive one as the series studied was small. This was due to insufficient publicity being given to the setting up of the Encephalitis Unit so that all cases of encephalitis were not referred to the Lady Hardinge Hospital.

2. The inability to carry out E.E.G. studies on all cases was also another regrettable feature.

3. Cases which had been studied prior to the formal institution of the enquiry (4 children and 4 adults) have also been included in this report.

19 children and 7 adult women were studied in this enquiry during the epidemic. 6 children and 3 adults died giving an overall mortality of 28.9 per cent.

CHILDREN

Onset.

The ages of the children ranged from 8 months to 11 years. The onset was ushered in by the following symptoms in order of frequency (i) fever (ii) twitchings of face and limbs (iii) vomiting and diarrhoea (iv) coma (v) abnormal behaviour.

The temperature on admission ranged between 99–104°F and the total pyrexial period from 2-16 days. In fatal cases there was terminal hyperpyrexia (upto 107° F).

Neurological findings :

Consciousness was disturbed in 14 cases out of 15 and 4 were deeply unconscious. Superficial and deep reflexes were extremely variable as was power and tone in the muscles. The cranial nerves were normal in all except one who had a divergent squint. Abnormal movements notice were of choreiform athetotic and ballismic types, eating automatism, oculogyric crises, localised and generalised twitchings (resembling myoclonus and a peculiar abduction spasm of both arms in one case.

E.E.G. was done in 4 cases. 3 showed well marked fast activity i.e. 25-30 cycles/sec. all over the Cortex and one slow wave with a frequency of 2-3/sec.

Other findings :

2 cases developed herpangina, one on the 4th day of illness and the other 3 weeks after the temperature settled down.

Laboratory data :

Cerebro spinal fluid.—The only significant findings were low sugar and low proteins in the majority of the cases.

Blood.—A leucocytosis was present in half the cases.

Virus Studies.—Blood for virus immunological reactions was taken from all the cases. Stools and C.S.F. were also sent for virus culture. Brain biopsy was done in one case. The reports on the virus studies are awaited.

ADULTS

7 adults (women) were studied in this series. Their ages ranged from 20-31 years. The onset was marked by fever, twitchings or unconsciousness. The total pyrexial period varied from 2—18 days and terminal hyperpyrexia was noted in the fatal cases.

Neurological findings :

Consciousness was disturbed in all cases, although only 2 cases were deeply unconscious. Neck rigidity was present in 2 cases and Kernig's sign in one case. Aphasia was present in one case.

Cranial nerve involvement included infranuclear type facial paralysis and convergent squint. Abnormal movements noticed were twitchings (usually localised) and static tremor.

Laboratory findings :

Cerebro spinal fluid.—Pleocytosis was noticed in 2 cases. Autopsy was done on one case. There were no macroscopic abnormalities.

Blood, C.S.F. and stools were sent for virus studies and the reports are awaited.

COMMENT

1. The unusual features of this epidemic was the presence of automatic feeding movements and oculogyric crises which have not been reported previously in Indian epidemics.

2. Another unusual feature was the development of herpangina in 2 of the cases (children) with encephalitis. There was also an outbreak of herpangina at Najafgarh in May and an outbreak of foot and mouth disease among cattle in the same locality in April and May. Whether these diseases were interrelated, it is difficult to tell.

IV. INDIAN JOURNAL OF MEDICAL RESEARCH

The Indian Journal of Medical Research is published by the Indian Council of Medical Research quarterly in January, April, July and October each year. The Journal is being published since 1913 and has throughout maintained a high standard. Papers based on original work and also articles of a clinical nature are accepted for publication in the Journal. It is one of the chief medium for the dissemination of information on medical research work carried out in India under the aegis of the Council and elsewhere.

There are about 300 subscribers to the Journal and about 170 copies are exchanged with other Journals published in India and abroad. The Journal is appearing regularly every quarter. In recent years the number of papers received for publication in the Journal has considerably increased and it has not been possible to accommodate all the papers in the quarterly number. With a view to publish promptly all the papers it has been decided to publish the Journal bimonthly from the next volume of the Journal to be published in January, 1958.

V. INDIAN JOURNAL OF MALARIOLOGY

The Indian Journal of Malariology is published quarterly by the Council in March, June, September and December each year. The Journal successfully completed 25 years of its service to the malaria world in 1955. The Silver Jubilee of the Journal was celebrated in December 1955 and on this occasion a special Silver Jubilee Number was brought out in which workers both from India and abroad contributed papers. It was very well received and deeply appreciated by malaria workers.

The Journal serves to disseminate new knowledge in particular in the field of malariology and on those diseases which are caused by mosquitoes and other arthropod borne vectors and generally on entomology and parasitology.

It has very wide circulation both in India and abroad. It has exchange relationship with 114 Journals. The annual subscription of the Journal consisting of four numbers is Rs.12 per volume and the price of a single copy of each quarterly number is Rs. 5.

VI. MICROFILM & PHOTOCOPY SERVICE UNITS

The Indian Council of Medical Research have established two microfilm & photocopy service units, one at the Central Research Institute, Kasauli and the other at the Indian Cancer Research Centre, Bombay, with a view to make available to Scientific Workers copies of important references from journals and periodicals which are otherwise inaccessible or cannot be procured readily. Copies are supplied at cost price as microfilms for the reading of which a 'Microfilm Reader' is required or as Photocopy which can be read with the naked eye without the assistance of any special appliance.

During the year under report the unit at the Central Research Institute, Kasauli, supplied to medical institutions and research workers in India and abroad 1192 pages of microfilms and 721 pages of photostat copies. This unit also supplied 29 laboratory photographs and six photomicrographs.

The unit at Bombay supplied 2875 pages of microfilms and 1297 pages of photocopies to various medical colleges and research institutions in India.

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VIII. LIST OF PAPERS ON WORK DONE UNDER THE AUSPICES OF THE INDIAN COUNCIL OF MEDICAL RESEARCH PUBLISHED DURING THE YEAR 1956

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